Literature Reviews

Ammonia-N concentration in alfalfa silage and its effects on dairy cow performance: A meta-analysis¤

Concentración del nitrógeno amoniacal en el ensilaje de alfalfa y sus efectos sobre el desempeño de vacas lecheras: Meta-análisis

Concentração de nitrogênio amoniacal em silagem de alfafa e seus efeitos sobre o desempenho de vacas leiteiras: Meta-análise

Juan I Sánchez-Duarte[*], PhD(c); Álvaro García, DVM, PhD.

Dairy and Food Science Department, South Dakota State University, Brookings 57007, USA.

Abstract

Background: Ammonia nitrogen (N) in alfalfa silage is used to evaluate silage quality, but its effect on dairy cows production is unknown. **Objective:** A meta-analysis was conducted to assess the effects of ammonia-N concentration in alfalfa silage on dairy cow performance. **Methods:** Thirty-four comparisons from 10 studies were analyzed with linear and quadratic models by the mixed-effect models of the R program using study as a random effect. The variance explained by the models was evaluated calculating marginal $R^2_{(m)}$ and conditional $R^2_{(c)}$. **Results:** Dry matter intake declined (DMI; p = 0.007), and milk production and milk fat yield tended to decrease linearly in response to increasing ammonia-N concentration (p = 0.08). Milk fat and milk protein percentages were unaffected. Milk protein yield, however, showed a positive linear trend effect (p = 0.07) along with a significant quadratic effect (p = 0.01) in response to increasing ammonia-N concentration. Values of $R^2_{(m)}$ and $R^2_{(c)}$ indicated less variation for significant models. **Conclusion:** Increased ammonia-N in alfalfa silage reduced DMI, negatively impacting milk production and milk fat yield. The quadratic response in milk protein yield might be explained by the initial reduction in milk production, and the subsequent utilization of ammonia-N to produce more rumen microbial protein, which results in more milk protein.

Keywords: *dairy cattle, Medicago sativa L., milk fat, milk protein, milk yield, regression model.*

Resumen

Antecedentes: El nitrógeno (N) amoniacal en el ensilaje de alfalfa es utilizado para evaluar la calidad del ensilaje, pero se desconoce su efecto sobre la producción en vacas lecheras. **Objetivo:** Se realizó un meta-análisis para evaluar los efectos de la concentración del N amoniacal en el ensilaje de alfalfa sobre el desempeño de vacas lecheras. **Métodos:** Treinta y cuatro comparaciones de 10 estudios fueron analizadas utilizando modelos lineales y cuadráticos mediante modelos de efectos mixtos de R, incluyendo el estudio como efecto aleatorio. La varianza explicada por los modelos se evaluó calculando la $R^2_{(m)}$ marginal y la $R^2_{(c)}$ condicional. **Resultados:** El consumo de materia seca se redujo (DMI; p = 0,007), y la producción de leche y grasa en leche tendieron a disminuir linealmente (p = 0,08) en respuesta al incremento en la concentración de N amoniacal en el ensilaje. Los porcentajes de grasa y proteína en leche no fueron afectados. La producción de la proteína en leche, sin embargo, mostró una tendencia de efecto lineal positivo (p = 0,07) junto a un efecto cuadrático significativo (p = 0,01) al aumentar el N amoniacal del ensilaje. Los valores de $R^2_{(m)}$ y $R^2_{(c)}$ indicaron menor variación en los modelos significativos. **Conclusión:** El incremento del N amoniacal en el ensilaje de alfalfa redujo el DMI, la producción de leche y la producción de grasa en leche. La respuesta cuadrática en la producción de proteína en leche puede ser explicada por la reducción inicial en producción de leche, con el uso subsecuente a nivel ruminal del N amoniacal para producir más proteína microbiana, lo cual resulta en mayor proteína en leche.

Palabras clave: *ganado lechero, grasa láctea, Medicago sativa L., modelo de regresión, producción de leche, proteína láctea.*

Resumo

Antecedentes: O nitrogênio amoniacal na silagem de alfafa é utilizado para avaliar a qualidade da silagem, porém o seu efeito sobre a produção de vacas leiteiras é desconhecido. **Objetivo:** Realizar uma meta-análise para avaliar os efeitos da concentração de nitrogênio amoniacal na silagem de alfafa no desempenho de vacas leiteiras. **Métodos:** Trinta e quatro comparações compiladas a partir de dez trabalhos de pesquisa foram analisadas usando modelos mistos com coeficiente linear e quadrático do programa R, e considerando o estudo como efeito aleatório. A variância explicada do modelo foi avaliada calculando a distribuição marginal $R^2_{(m)}$ e a condicional $R^2_{(c)}$. **Resultados:** Houve redução no consumo de matéria seca (DMI; p = 0,007), e uma tendência em reduzir linearmente a produção de leite e de gordura em resposta ao aumento da concentração de nitrogênio amoniacal na silagem (p = 0,08). As porcentagens de gordura e proteína do leite não foram afetadas. No entanto, houve uma tendência linear (p = 0,07), acompanhada de um significativo efeito quadrático (p = 0,01) para o rendimento de proteínas do leite em resposta ao aumento do nitrogênio amoniacal na silagem de alfafa. Os valores de $R^2_{(m)}$ e $R^2_{(c)}$ indicaram menor variação para os modelos significativos. **Conclusão:** O aumento do nitrogênio amoniacal na silagem de alfafa resultou na redução do DMI, afetando negativamente a produção de leite e o teor de gordura no leite. A resposta quadrática para o rendimento de proteínas do leite pode ser explicada pela redução inicial da produção de leite e à subsequente utilização do nitrogênio amoniacal presente na silagem para produzir mais proteína microbiana sintetizada a nível ruminal e consequentemente, mais proteínas do leite.

Palavras chave: *gado leiteiro, gordura do leite, Medicago sativa L., modelo de regressão, produção de leite, proteínas do leite.*

Introduction

Alfalfa is perhaps one of the most beneficial forages grown in dairy farms because of its high nutritional quality (Broderick, 1995) and its ability to fix soil nitrogen (Russelle, 2004). Alfalfa forage, however, is difficult to ensile because of its high crude protein content (Muck, 1988), low soluble carbohydrate concentrations (Owens *et al.*, 1999) and high buffering capacity (Muck and Walgenbach, 1985). These characteristics, combined with inadequate DM content and difficult compaction during ensiling, lead to production of silages with high ammonia-N concentration (Muck, 2011).

Ammonia-N concentration in alfalfa silage has sparked interest as an indicator to evaluate silage quality; however, its potential utilization by the cow

is still debated. High degradation rates of protein into silage ammonia-N contribute usually to increase rumen ammonia concentrations (Givens and Rulquin, 2004). Rumen ammonia is recovered in the crude protein fraction together with amino acids and peptides; however, the site of utilization in the cow differs (Buchanan-Smith and Yao, 1978). In fact, rumen ammonia is an essential nutrient for fiber-digesting microorganisms, although the extent of its utilization varies depending on the amount and solubility of the carbohydrates present in the rumen (Van Soest, 1994). Some studies have reported the influence of ammonia-N in grass silage on dairy cow performance. Van Os *et al.* (1995) demonstrated a significant DMI reduction and a trend for decreased milk production in cows fed grass silages with 13 vs 6% ammonia-N. In their study, rumen ammonia was higher in cows fed silage with 13% ammonia-N. Dry matter intake dropped 0.3 Kg/d when ammonia-N in grass silage fed to dairy cows increased from 7 to 20% (Wright *et al.* 2000). The analysis of 47 experiments indicated that high concentrations of ammonia-N in grass silage fed to dairy cows had a negative effect on DMI, milk production and milk composition (Huhtanen *et al.*, 2003).

Although high ammonia-N concentrations in grass silage have been demonstrated to reduce milk production, there is limited information on the effects of its presence in alfalfa silage fed to dairy cows. Therefore, the objective of this study was to evaluate through a meta-analysis the effects of the concentration of ammonia-N in alfalfa silage on the performance of lactating dairy cows.

Materials and methods

A literature search was performed in PubMed, Google Scholar, and Agricola-USDA using the keywords "alfalfa silage" and "dairy cows". This search was complemented by using the citations in scientific papers for a more comprehensive database. A total of 59 papers were found, but only 10 (Kung *et al.*, 1987; Kent *et al.*, 1988; Kung *et al.*, 1989; Fredeen *et al.*, 1991; Nagel and Broderick, 1992; Colombari *et al.*, 2001; Kung *et al.*, 2003; Broderick *et al.*, 2007; Krizsan *et al.*, 2007; Broderick and Muck, 2009) met the following criteria: 1) diets including alfalfa silage were statistically compared; 2) ammonia-N

concentration in alfalfa silage was clearly reported; 3) similar percentage of alfalfa silage was maintained across diets; 4) alfalfa silage was not mixed with other forage sources; 5) alfalfa silage was harvested at the same stage of maturity; 6) physical form of alfalfa silage was not altered in the diets; and 7) individual lactating dairy cows were used as experimental units to evaluate the effects of alfalfa silage.

The studies reported by Kung *et al.* (1987; 1989) were evaluated as separate experiments since alfalfa silage was from two different crop years, resulting in different silages and cow information. In all studies production analysis included fat-corrected milk. One study was not considered in the analysis of milk fat and milk protein percentages due to lack of information (Fredeen *et al.*, 1991). Other studies did not include milk fat and milk protein yields (Kung *et al.*, 1987; Kent *et al.*, 1988; Kung *et al.*, 1989), and were thus calculated from their milk fat and milk protein percentages.

Data for DMI, milk production, milk component percentages and yields were weighted according to their standard errors as proposed by St-Pierre (2001). In the three studies where milk fat and protein yields were calculated, a standard error from their respective milk fat and protein percentages was considered. The assessment of ammonia-N concentration in alfalfa silage on cow performance was evaluated by the mixed-effect models approach of R program (R Core Team, 2015); specifically, the nlme package was used (Pinheiro *et al.*, 2016). Linear and quadratic effects of ammonia-N in alfalfa silage were modeled using study as a random effect (St-Pierre, 2001) according to the following models:

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + S_i + b_i X_{ij} + \varepsilon_{ij}$$
$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + \beta_2 X_{ij}^2 + S_i + b_i X_{ij} + \varepsilon_{ij}$$

Where:

Y_{ij} = dependent variable representing DMI, milk production or milk composition at level j of the variable X in the study i.

β_0 = overall fixed intercept across studies.

β_1 and β_2 = the overall fixed regression model coefficients of Y on X across studies.

X_{ij} = independent variable representing ammonia at level j of the variable X in the study i.

S_i = random effect of study i ($i = 10$).

b_i = random effect of study i on the regression coefficient of Y on X in study i.

ε_{ij} = unexplained residual error.

When a quadratic effect was statistically significant, a break point was estimated in the adjusted data to determine the inflection point in which the response variable changes. The prediction was tested using piecewise generalized mixed models with the lme4 package (Douglas *et al.*, 2015) with a break point range between 0.17 and 10.94% ammonia-N. The lowest Akaike Information Criterion (AIC; Akaike, 1974) and the level of significance ($p<0.05$) were used for selecting the best fitted broken-line regression model.

Since the analysis was performed using mixed effect models, it was necessary to calculate the marginal $R^2_{(m)}$ (proportion of variance explained by the fixed factor) and conditional $R^2_{(c)}$ (proportion of variance explained by both fixed and random factors) to quantify the goodness-of-fit of the models according to Nakagawa and Schielzeth (2013). Evaluating goodness-of-fit of a model generated by the mixed model procedure with the simple R^2 does not account for the proofs of the asymptotic distributions of the cumulative sum and the consistence of the tests derived from the random effects (Pana and Lin, 2005).

Heteroscedasticity of the models was evaluated by checking the residual vs fitted values plot and normality through the Q-Q plot. Significance was set at $p<0.05$ for all variables evaluated, and trends were established at $0.05<p \leq 0.10$.

Results

The database included mostly 34 comparisons reported in 10 papers published from 1987 to 2009 (Table 1). Ammonia-N concentration in alfalfa silage averaged 5.20% across studies with a range between 0.17 and 10.94%. Substantial ranges were also observed for DMI, milk production and milk components. Similar mean and median values for all variables indicated that data was normally distributed.

Parameter estimates for linear and quadratic mixed effect models are shown in Table 2. Outcomes of the current meta-analysis indicated that DMI declined ($p = 0.0007$) and milk production tended to decrease linearly ($p = 0.08$) in response to the linear function of increasing ammonia-N concentration in alfalfa silage.

Coefficients of prediction equations for DMI and milk production suggested that for every additional percentage of ammonia-N in alfalfa silage, DMI and milk production can be reduced 0.42 and 0.47 Kg/d, respectively (Figures 1 and 2). Marginal R^2 for DMI and milk production were 0.31 and 0.07, whereas conditional R^2 for both models were 0.92 and 0.89, respectively.

Increasing ammonia-N in alfalfa silage did not affect milk fat nor protein percentages (Table 2), although there was a trend to decrease milk fat ($p = 0.08$) and protein yields ($p = 0.07$); it is important to note, however, that milk protein yield was best fitted to a quadratic regression model.

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

Variable	n^1	Mean	Median	SD	Minimum	Maximum
Ammonia-N in silage (%)	34	5.20	4.92	2.83	0.17	10.94
DMI, Kg/d	34	22.39	23.05	2.06	18.10	25.40
Milk production, Kg/d	34	31.92	31.35	5.85	24.40	42.24
Milk fat, %	31	3.67	3.67	0.25	3.13	4.20
Milk fat yield, Kg/d	34	1.16	1.16	0.20	0.84	1.53
Milk protein, %	31	3.09	3.07	0.19	2.68	3.43
Milk protein yield, Kg/d	34	0.99	1.01	0.15	0.76	1.35

[1]Number of experimental treatments; SD: Standard deviation.

Table 2. Linear and quadratic regression models for DMI, milk yield, milk fat, and milk protein to assess ammonia-N concentration in alfalfa silage and its effects on the performance of lactating dairy cows.

Model	Coefficients					
Linear model	**Intercept**	**SE**	**p-value**	**NH$_3$-N**	**SE**	**p-value**
DMI, Kg/d	24.551	0.750	0.000	-0.422	0.105	0.0007
Milk production, Kg/d	33.682	1.938	0.000	-0.474	0.262	0.086
Milk fat, %	3.646	0.137	0.000	0.007	0.023	0.749
Milk fat yield, Kg/d	1.243	0.074	0.000	-0.020	0.011	0.083
Milk protein, %	3.139	0.090	0.000	-0.003	0.014	0.802
Milk protein yield, Kg/d	1.045	0.055	0.000	-0.014	0.007	0.070

| **Quadratic model**	**Intercept**	**SE**	**p-value**	**NH$_3$-N**	**SE**	**p-value**	**NH$_3$-N^2**	**SE**	**p-value**
DMI, Kg/d	24.988	1.087	0.000	-0.619	0.374	0.114	0.017	0.031	0.591
Milk production, Kg/d	37.365	2.632	0.000	-2.037	0.901	0.036	0.130	0.075	0.101
Milk fat, %	3.512	0.207	0.000	0.078	0.085	0.370	-0.007	0.008	0.392
Milk fat yield, Kg/d	1.371	0.105	0.000	-0.078	0.037	0.052	0.005	0.003	0.133
Milk protein, %	3.167	0.141	0.000	-0.017	0.054	0.759	0.001	0.005	0.800
Milk protein yield, Kg/d	1.192	0.070	0.000	- 0.077	0.024	0.005	0.005	0.002	0.018

SE: Standard error; NH-N$_3$: Ammonia-N.
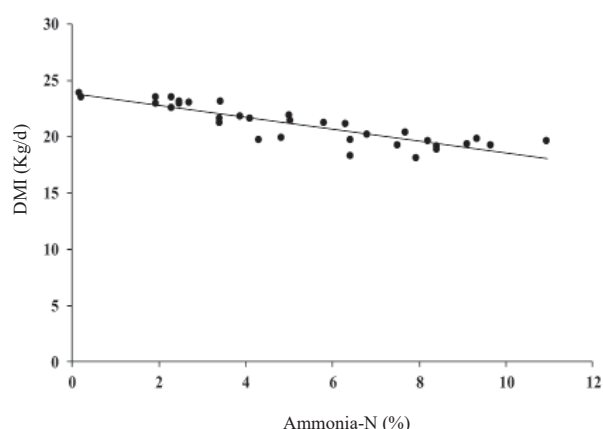
Figure 1. Linear DMI response to increasing ammonia-N concentration in alfalfa silage. Plot reports 34 observations adjusted for the random effect of trial. DMI = 24.551 – 0.422 × ammonia-N ($R^2_{(m)}$ = 0.311, $R^2_{(c)}$ = 0.924, p = 0.0007).
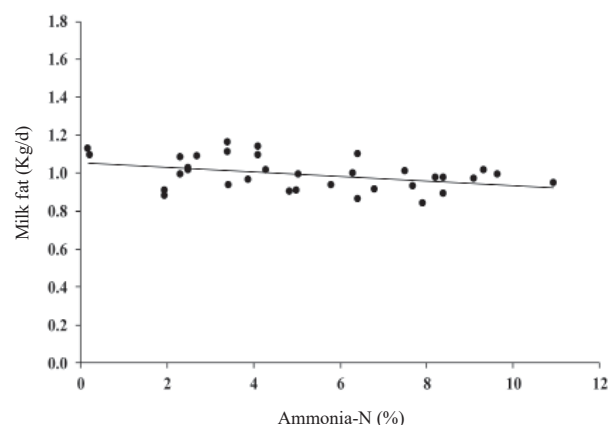

Figure 2. Linear milk production response to increasing ammonia-N concentration in alfalfa silage. Plot reports 34 observations adjusted for the random effect of trial. Milk production = 33.682 – 0.474 × ammonia-N ($R^2_{(m)}$ = 0.070, $R^2_{(c)}$ = 0.894, p = 0.086).

Similar to DMI and milk production models, a decrease of 0.02 Kg/d milk fat is expected for each percentage point increase in ammonia-N in alfalfa silage (Figure 3). Marginal and conditional R^2 for the milk fat yield model were 0.11 and 0.85, respectively.

The quadratic responses related to DMI, milk production, milk fat and protein percentage, and milk fat yield were not altered by increasing ammonia-N concentration in alfalfa silage (p>0.05; Table 2). However, milk protein yield responded quadratically to the increase in ammonia-N of alfalfa silage (p = 0.01). According to the quadratic broken-line regression model, milk protein yield was reduced 0.077 Kg/d until ammonia-N in alfalfa silage was 6.81%, then milk protein yield increased 0.005 Kg/d (Figure 4). Marginal and conditional R^2 for the milk protein yield model were 0.30 and 0.90, respectively.
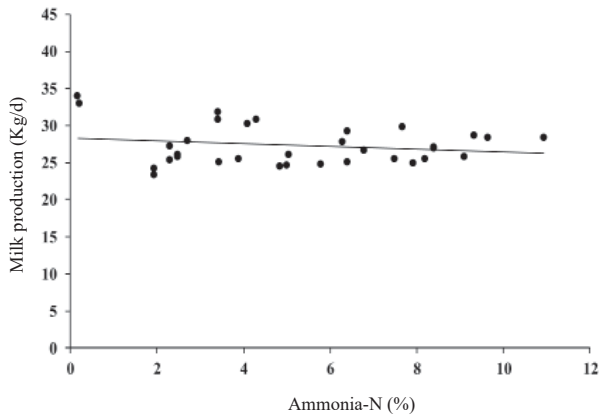
Figure 3. Linear milk fat yield response to increasing ammonia-N concentration in alfalfa silage. Plot reports 34 observations adjusted for the random effect of trial. Milk fat yield = $1.243 - 0.020 \times$ ammonia-N ($R^2_{(m)} = 0.105$, $R^2_{(c)} = 0.854$, $p = 0.083$).

Figure 4. Quadratic milk protein yield response to increasing ammonia-N concentration in alfalfa silage. Plot reports 34 observations adjusted for the random effect of trial. Milk protein yield = $1.192 - 0.077 \times$ ammonia-N + $0.005 \times$ ammonia-N^2 ($R^2_{(m)} = 0.296$, $R^2_{(c)} = 0.902$, $p = 0.018$). Break point = 6.81% ammonia-N (Estimate = -0.0077, SE = 0.0029, $p < 2e-16^{***}$, AIC = -81.77).

The statistical differences and trends observed in linear and quadratic models indicate that after adjusting for differences between studies, ammonia-N concentration in alfalfa silage significantly contributed to the analysis of the productive variables in this meta-analysis. Marginal and conditional values were higher in significant models compared to models with significant trends, indicating that there was more variability associated to the random effect along with residuals and the variance distribution in models with significant trends.

The diagnostic of the heteroscedasticity and normality for the models shown in Figures 1, 2, 3, and 4 are presented in Figures 5 and 6. Residuals vs fitted plots for DMI (Figure 5a), milk production (Figure 5b), and milk fat yield (Figure 6c) show that residuals are symmetrical and their errors are, at least, normally distributed. In addition, the Q-Q plots of these models indicate that residuals followed a straight line, so errors for DMI, milk production, and milk fat yield models belong to a normal distribution. However, residual vs fitted plot and Q-Q plot of the milk protein yield model (Figure 6d) indicates that residuals are asymmetrical and deviated from the straight line, respectively. Therefore, it was demonstrated that a linear model is not a good fit for this variable.
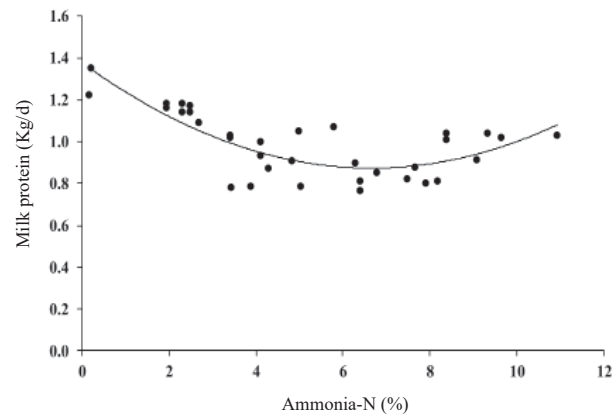
Discussion

Information of increasing ammonia-N contents in alfalfa silage and productive parameters were analyzed by a meta-analytical procedure in an effort to elucidate the effect of ammonia-N concentration on dairy cow performance. To accomplish this goal, data from published papers were analyzed using study as a random effect and weighted observations to account for the heterogeneity across studies. St-Pierre (2001) indicated that ignoring the study effect and avoiding weighting the observations would severely skew the estimated parameters and affect the normal distribution of the residual errors in the regression models, respectively. In addition, this meta-analysis used R^2 for mixed-effect models for explaining the variance of the models (Nakagawa and Schielzeth, 2013), and heteroscedasticity and normality were evaluated through a graphical method. It has been demonstrated that marginal and conditional R^2 are the best statistics to quantify the variance explained in a model when the mixed-model procedure is utilized (Nakagawa and Schielzeth, 2013; Johnson, 2014). Besides, the assessment of heteroscedasticity and normality by residual plots guarantee that coefficients of the regression model are not being biased (Altman and Krzywinsky, 2016). Therefore, findings in this meta-analysis are interpreted with a good degree of reliability.
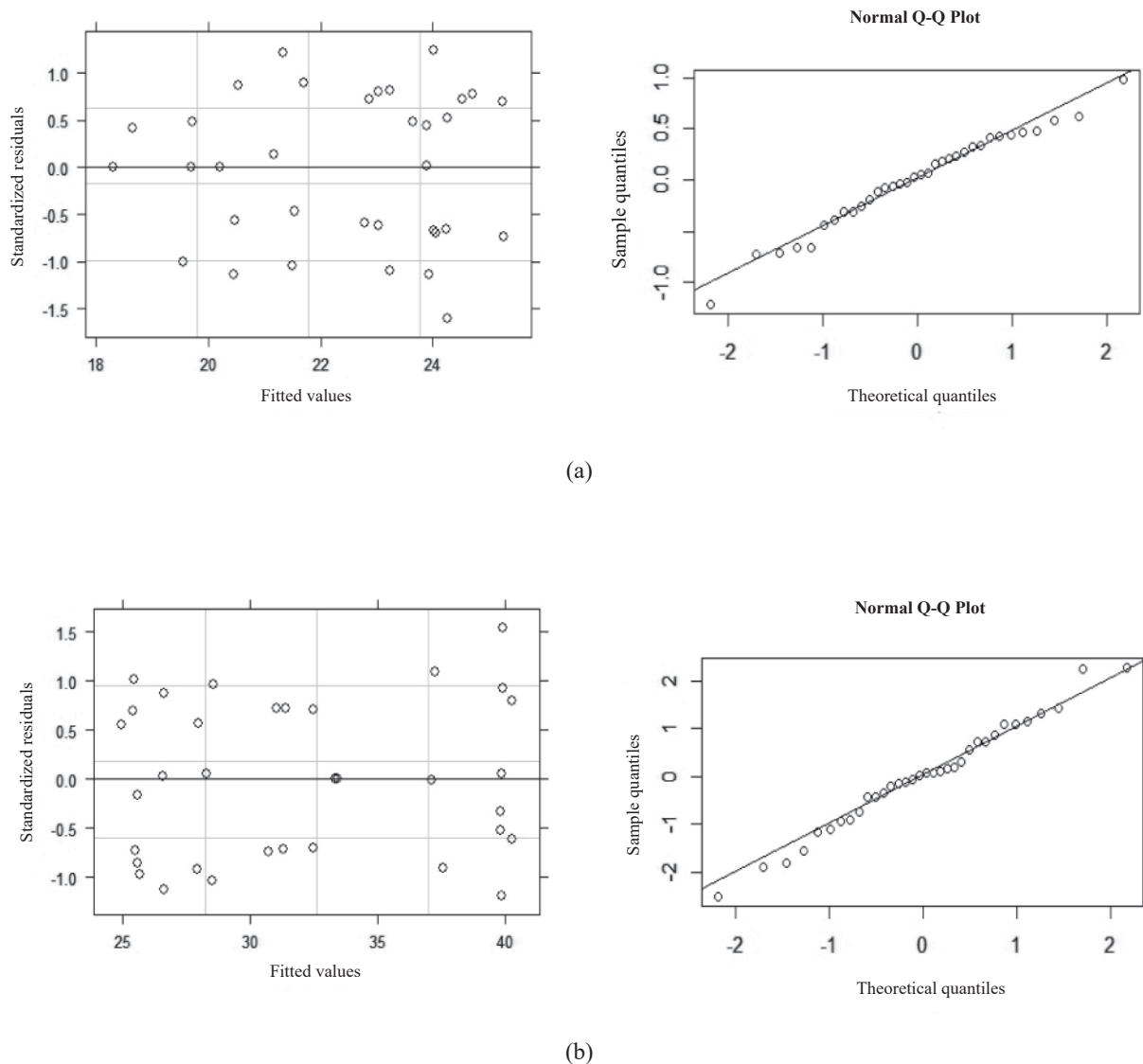
(a)

(b)

Figure 5. Residuals vs fitted and Q-Q plots for DMI (a) and milk production (b).

Ammonia-N in silages results from protein breakdown by plant proteolytic enzymes or clostridial microorganisms (Ohshimaa and McDonald, 1978). An intensive clostridial action or a slow drop of pH in the silo can be responsible for producing silages with more than 12% ammonia-N concentration (Kung and Shaver, 2001). Ammonia-N concentrations of alfalfa silages used in this meta-analysis were not greater than 12%. However, even concentrations from 0.2 to 11%, showed a negative impact on DMI (Figure 1). The specific mechanism by which ammonia-N affects DMI is not well understood.

Ammonium salt injected in goats (Conrad *et al.*, 1977) or urea dosed in the rumen of Holstein steers (Bartley *et al.*, 1981) resulted in cellular acidification and high ammonia concentration in the carotid artery, respectively, which affected the central nervous system and reduced feed intake. In this meta-analysis milk production just tended to decrease linearly in response to increasing ammonia-N concentration in alfalfa silage (Figure 2). In dairy cows, a depression of DMI reduces nutrient uptake, and decreases milk production since intake is the single most important factor affecting it (Weiss, 2015). Therefore, the
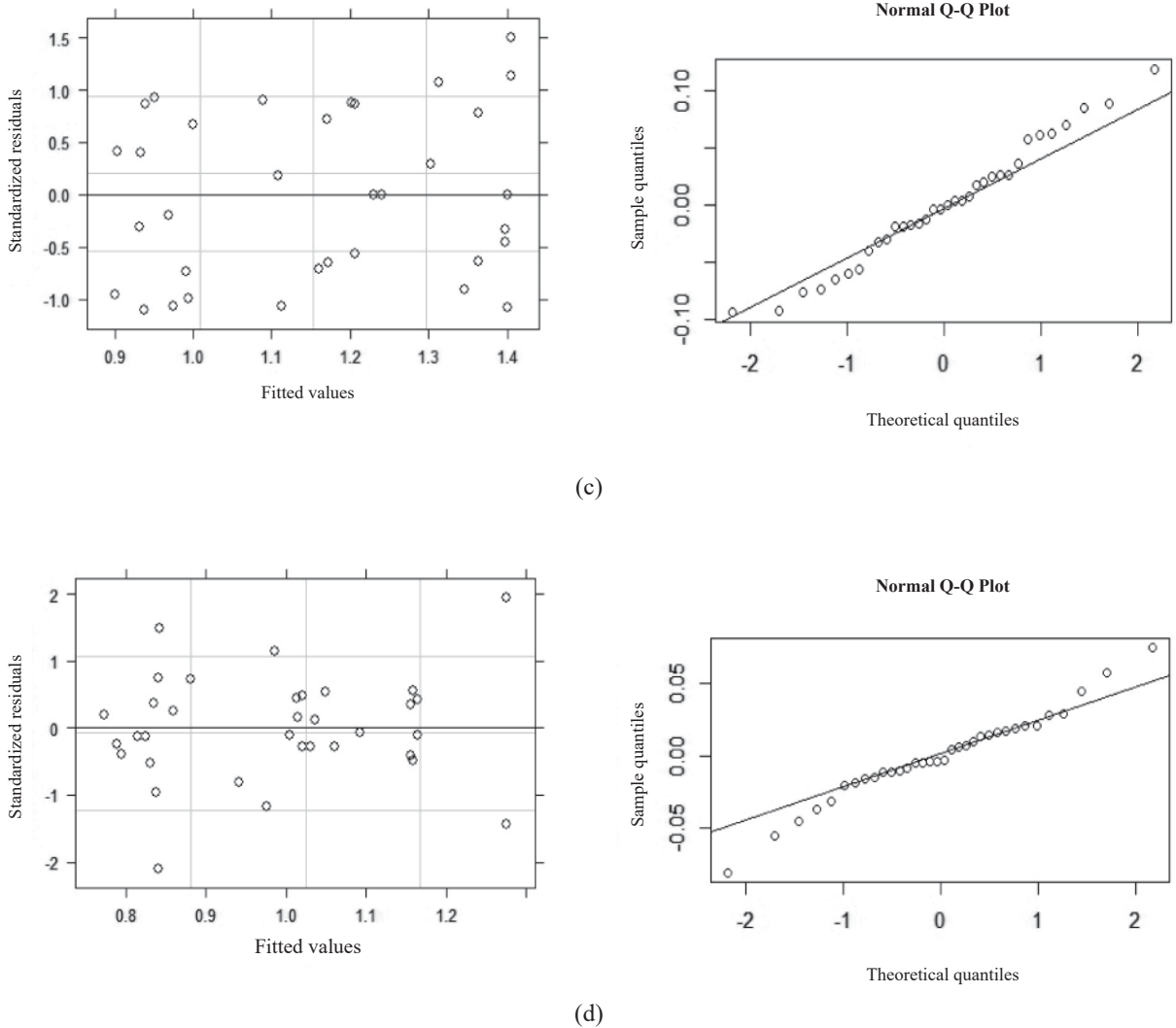
Figure 6. Residuals vs fitted and Q-Q plots for milk fat yield (c) and milk protein yield (d).

inverse weak relationship between ammonia-N and milk production observed in this meta-analysis may be due to the ammonia-N concentration in alfalfa silage indirectly reduced milk production through a DMI depression. Similar to the findings of this meta-analysis, Van Os *et al*. (1995) reported DMI was reduced 0.8 Kg/d and milk production tended to decrease 2.2 Kg/d, in response to an increase in grass silage ammonia-N from 6 to 13%.

Milk fat and protein percentages were not affected by ammonia-N concentration in alfalfa silage (Table 2). However, increased ammonia-N in silage tended to decrease milk fat (Figure 3) and protein

yields, but with a highly significant quadratic effect on milk protein yield (Figure 4). Huhtanen *et al*. (2003) found that ammonia-N concentration in grass silage is negatively correlated with the percentages and yields of milk fat and protein in dairy cows. They also observed that for milk protein yield a quadratic effect had a slightly better fit than a linear model. In the current meta-analysis, milk fat yield may be reduced because milk production declines in response to ammonia-N concentration in alfalfa silage. The quadratic response of milk protein yield might be related to a combined effect of decreased milk production and the incorporation of ammonia-N from silage (>6.81% ammonia-N in this meta-analysis)

into microbial protein or into the N flowing to the small intestine. It has been reported that soluble N from grass silages escapes ruminal degradation and contributes up to 10% to the intestinal amino acids supply in dairy cows (Volden *et al.*, 2002), which can be later used for milk protein synthesis in the mammary gland (Doepel *et al.*, 2002). Nevertheless, there is not firm evidence that high ammonia-N from silage contributes to milk protein synthesis through its integration into the pool of N used by the cow.

In conclusion, prediction models in this meta-analysis demonstrated that increased ammonia-N in alfalfa silage had a negative linear effect on DMI, but tended to reduce milk production, milk fat and protein yields. Milk production may be indirectly affected because DMI was reduced and milk fat yield declined in response to decreased milk production. Additionally, increasing ammonia-N in alfalfa silage had a significant quadratic effect on milk protein yield, which might be related to the reduced milk production and a possible contribution of high ammonia-N from silage to the nitrogen used by rumen bacteria, which is later used by the cow to produce milk protein.

Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

References

Akaike H. A new look at the statistical model identification. IEEE Trans Autom Control 1974; 19:716-723.

Altman N, Krzywinsky M. Points of significance: Regression diagnostic. Nature Met 2016; 13:385-386.

Bartley EE, Avery TB, Nagaraja TG, Watt BR, Davidovich A, Galitzer S, Lassman BL. Ammonia toxicity in cattle. V. Ammonia concentration of lymph and portal, carotid, and jugular blood after the ingestion of urea. J Anim Sci 1981; 53:494-498.

Broderick GA, Brito AF, Olmos Colmenero JJ. Effects of feeding formate-treated alfalfa silage or red clover silage on the production of lactating dairy cows. J Dairy Sci 2007; 90:1378-1391.

Broderick GA, Muck RE. Effect of alfalfa silage storage structure and rumen-protected methionine on production in lactating dairy cows. J Dairy Sci 2009; 92:1281-1289.

Broderick GA. Performance of lactating dairy cows fed either alfalfa silage or alfalfa hay as the sole forage. J Dairy Sci 1995; 78:320-329.

Buchanan-Smith JG, Yao YT. Non-protein nitrogen in corn silage: a partial characterization, its utilization in the rumen and effect upon digestibility and retention of nitrogen in lambs. Can J Anim Sci 1978; 58:681-690.

Colombari G, Borreani G, Crovetto GM. Effect of ensiling alfalfa at low and high dry matter on production of milk used to make grana cheese. J Dairy Sci 2001; 84:2494-2502.

Conrad HR, Baile CA, Mayer J. Changing meal patterns and suppression of feed intake with increasing amounts of dietary nonprotein nitrogen in ruminants. J Dairy Sci 1977; 60:1725-1733.

Doepel L, Pacheco D, Kennelly JJ, Hanigan MD, Lopez IF, Lapierre H. Milk protein synthesis as a function of amino acid supply. J Dairy Sci 2002; 87:1279-1297.

Douglas B, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Software 2015; 67:1-48.

Fredeen HA, McQueen RE, Browning DA. Effects of enzymes and nutrients in a bacterial inoculant on quality of timothy or alfalfa silage and dairy cow performance. Can J Anim Sci 1991; 71:781-791.

Givens DI, Rulquin H. Utilisation by ruminants of nitrogen compounds in silage-based diets. Anim Feed Sci Tech 2004; 114:1-18.

Huhtanen P, Nousiainen JI, Khalili H, Jaakkola S, Heikkila T. Relationships between silage fermentation characteristics and milk production parameters: Analyses of literature data. Livest Prod Sci 2003; 81:57-73.

Johnson PCD. Extension of Nakagawa & Schielzeth's R^2_{GLMM} to random slope models. Met Ecology Evol 2014; 5:944-946.

Kent BA, Arambel MJ, Walters JL. Effect of bacterial inoculant on alfalfa haylage: Ensiling characteristics and milk production response when fed to dairy cows in early lactation. J Dairy Sci 1988; 71:2457-2561.

Krizsan SJ, Broderick GA, Muck RE, Promkot C, Colombini S, Randby AT. Effect of alfalfa silage storage structure and roasting corn on production and ruminal metabolism of lactating dairy cows. J Dairy Sci 2007; 90:4793-4804.

Kung LJr, Craig WM, Satter LD. Ammonia-treated alfalfa silage for lactating dairy cows. J Dairy Sci 1989; 72:2565-2572.

Kung LJr, Satter LD, Jones BA, Genin KW, Sudoma AL, Enders GLJr, Kim HS. Microbial inoculation of low moisture alfalfa silage. J Dairy Sci 1987; 70:2069-2077.

Kung LJr, Shaver R. Interpretation and use of silage fermentation analysis reports. Focus on forage, 2001; Vol. 3, No. 13. University of Wisconsin Extension.

Kung LJr, Taylor CC, Lynch MP, Neylon JM. The effect of treating alfalfa with lactobacillus buchneri 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. J Dairy Sci 2003; 86:336-343.

Muck RE, Walgenbach RP. Variations in alfalfa buffering capacity. Am Soc Agric Eng 1985: ASAE Paper No. 85-1535.

Muck RE. Factors influencing silage quality and their implications for management. J Dairy Sci 1988; 71:2992-3002.

Muck RE. The art and science of making silage. Proceeding, Western Alfalfa & Forage Conference; 2011 Dec 11-13; Las Vegas, NV. Davis, CA.

Nagel SA, Broderick GA. Effect of formic acid or formaldehyde treatment of alfalfa silage on nutrient utilization by dairy cows. J Dairy Sci 1992; 75:140-154.

Nakagawa S, Schielzeth H. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Met Ecology Evol 2013; 4:133-142.

Ohshimaa M, McDonald P. A review of the changes in nitrogenous compounds of herbage during ensilage. J Sci Food Agric 1978; 29:491-505.

Owens VN, Albrecht KA, Muck RE, Duke SH. Protein degradation and fermentation characteristics of red clover and alfalfa silage harvested with varying levels of total nonstructural carbohydrates. Crop Sci 1999; 39:1873-1880.

Pan Z, Lin DY. Goodness-of-fit methods for generalized linear mixed models. Biometrics 2005; 61:1000-1009.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. _nlme: Linear and Nonlinear Mixed Effects Models_2016; R package version 3.1-128.

R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2015.

Russelle M. The environmental impact of N_2 fixation by alfalfa. Proceedings, National Alfalfa Symposium; 2004 Dec 13-15; San Diego, CA. UC, Davis 2004.

St-Pierre NR. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J Dairy Sci 2001; 84:741-755.

Van Os M, Dulphy JP, Baumont R, Jailler M, Ballet JM. The influence of ammonia and amines on grass silage intake and intake behavior in dairy cows. Ann Zootech 1995; 44:73-85.

Van Soest PJ. Nutritional ecology of the ruminant. 2nd ed. Cornell University Press; 1994. p.463.

Volden H, Mydland LT, Olaisen V. Apparent ruminal degradation and rumen escape of soluble nitrogen fractions in grass and grass silage administered intraruminally to lactating dairy cows. J Anim Sci 2002; 80:2704-2716.

Weiss B. Optimizing and evaluating dry matter intake of dairy cows. WCDS Adv Dairy Tech 2015; 27:189-200.

Wright DA, Gordon FG, Steen RWJ, Patterson DC. Factors influencing the response in intake of silage and animal performance after wilting of grass before ensiling: A review. Grass Forage Sci 2000; 55:1-13.