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Fermentative quality of silage as affected by protein level in the ensiled material: A meta-analysis

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Abstract. Ensiling of high-protein forages like alfalfa, indigofera, cassava and moringa, however, are characterized by considerable extent of proteolysis. This study aimed to perform a meta-analysis from various published experiments regarding the effect of protein level on fermentative quality of silage. Papers reported data on protein contents of silages and their fermentative characteristics were collected and integrated in a database. A total of 15 papers and comprised of 155 data points were included. The ensiled materials were peas, wheat, barley, maize, sorghum, alfalfa and mucuna. The ensiling period varied from 20 to 303 days. Data were statistically analyzed by using the mixed model methodology. Studies from different papers were treated as random effects whereas protein level in silage was treated as fixed effect. The model statistics used was p-value. Results revealed that higher protein level led to an increase of DM loss in silage ($p<0.01$). Protein was negatively correlated with NDF ($p<0.001$) and ADF ($p<0.05$). Increasing protein level elevated pH value of the silage ($p<0.05$) and tended to increase acetate concentration ($p<0.1$). Ethanol concentration was elevated by increasing silage protein level ($p<0.05$). Higher silage protein level increased organic matter digestibility (OMD), lactic acid bacteria (LAB) population, nitrate and ammonia concentrations of the silage ($p<0.05$). It can be concluded that higher protein level induces proteolysis in the silage and reduces its fermentative quality.

1. Introduction

Ensiling of feed materials is a common practice in livestock production. This technique is particularly important for conserving feeds when their production is high (during rainy season) for further use when feed production is limited (during dry season). Unlike hay-making, ensiling is less dependent on weather whereas the former requires a sunny weather for its successful application. Various forages can be conserved as silages such as grasses, legumes and agricultural by-products [1]. Often silage additives such as molasses, lactic acid bacteria inoculant, etc., are added to the ensiled materials in order to ensure a good quality of silage [2]. Ensiling of high-protein forages like alfalfa, indigofera, cassava and moringa, however, are characterized by considerable extent of proteolysis [3,4]. Protein in silage is subsequently degraded and deaminated to result amino acids and ammonia, respectively. This process ultimately reduces the quality of protein and may cause adverse effect on productive performance of livestock.

Despite the indication of proteolysis from high-protein forages has been repeatedly reported from a number of individual experiments, there is no study so far attempted to make a generalization on such phenomenon. This study therefore aimed to perform a meta-analysis from various published experiments regarding the effect of protein level on fermentative quality of silage. We hypothesized that higher level of protein in silage would lead to an enhanced proteolysis as indicated primarily by an increase of silage ammonia concentration.

2. Materials and methods

Papers reported data on protein contents of silages and their fermentative characteristics were collected and integrated in a database. Searching of the literatures was performed by using Scopus and Google Scholar databases. A total of 15 papers and comprised of 155 data points were included [5-19]. These were originated from various reputable journals such as Journal of the Science of Food and Agriculture, Animal Feed Science and Technology, Acta Veterinaria Brno, Industrial Crops and Products, Journal of Integrative Agriculture, and Journal of Dairy Science. The ensiled materials were peas, wheat, barley, maize, sorghum, alfalfa and mucuna. The ensiling period varied from 20 to 303 days. Parameters integrated were dry matter (DM) loss, chemical composition, silage fermentation characteristics, microbial population and organic matter digestibility (OMD). A summary statistics of the database is presented in table 1.

Table 1. Summary statistics of the database.

Parameter	Unit	n	Average	SD	Min	Max
CP	g/kg DM	155	106	49.4	56	250
DM loss	g/kg DM	44	48.6	38.4	0	133
Starch	g/kg DM	150	319	198	9.0	746
WSC	g/kg DM	126	37.2	68.4	0	355
NDF	g/kg DM	139	395	131	95	663
ADF	g/kg DM	135	233	83.8	37	379
Soluble N	g/kg N	22	607	143	430	878
pH	no unit	119	4.19	0.578	4.0	6.0
Lactate	g/kg DM	111	54.0	31.9	2.0	167
Acetate	g/kg DM	111	27.4	21.5	3.0	122
Propionate	g/kg DM	82	6.39	14.7	0	83
Butyrate	g/kg DM	61	5.35	18.7	0	98
Formate	g/kg DM	25	5.46	6.98	0	20
Ethanol	g/kg DM	74	16.1	12.4	1.0	61
OMD	g/kg DM	17	636	81.9	473	755
LAB	log cfu/g	52	8.40	2.29	2.0	12
Yeast	log cfu/g	73	14.3	23.0	1.0	81
Mould	log cfu/g	52	9.19	10.6	1.0	34
Nitrate	g/kg DM	12	0.70	0.578	0	2.0
NH_3	g/kg N	95	98.2	149	0	932

CP, crude protein; WSC, water soluble carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; OMD, organic matter digestibility; LAB, lactic acid bacteria; NH_3, ammonia; n, number of data; SD, standard deviation.

Data were statistically analyzed by using the mixed model methodology as described by St-Pierre [20]. The mathematical model was $Y_{ij} = B_0 + B_1X_{ij} + s_i + b_iX_{ij} + e_{ij}$, in which Y_{ij} = response variable, B_0 = overall intercept, B_1 = linear regression coefficient of Y on X (fixed effect), X_{ij} = value of the continuous predictor variable, s_i = random effect of experiment i, b_i = random effect of experiment i on the regression coefficient of Y on X in experiment i, and e_{ij} = random residual error. Studies from different papers were treated as random effects whereas protein level in silage was treated as fixed effect. Variable study was declared in class statement since it does not contain any quantitative information. No weighting procedure was applied in the current meta-analysis. The model statistics used was p-value; an effect was considered to be significant when $p<0.05$, and a tendency was declared when $0.05<p<0.1$. A positive slope indicated a positive effect (or relationship) of protein level on a certain parameter and *vice versa*. Computation of the statistical meta-analysis (proc mixed) was performed by using SAS software version 9.1.

3. Results and discussion

Higher protein level led to an increase of DM loss in silage ($p<0.01$; table 2). Protein had a negative relationship with starch, NDF and ADF contents ($p<0.05$), but it had lack of effect on WSC and soluble N fractions. Increasing protein level elevated pH value of the silage ($p<0.05$; table 3). It tended to increase acetate concentration ($p<0.1$), but had no effects on lactate, propionate, butyrate and formate concentrations. Ethanol concentration was elevated by increasing silage protein level ($p<0.05$). Higher silage protein level increased OMD, lactic acid bacteria (LAB) population, nitrate and ammonia concentrations of the silage ($p<0.05$; Table 4). Yeast and mould were not affected by protein level in silage.

Table 2. Effect of protein level on dry matter (DM) loss and chemical composition of silage.

Parameter	Unit	n	Intercept	Slope	p-value
DM loss	g/kg DM	44	0	0.456	0.003
Starch	g/kg DM	150	400	–0.692	<0.001
WSC	g/kg DM	126	55.5	–0.219	0.145
NDF	g/kg DM	139	516	–1.22	<0.001
ADF	g/kg DM	135	256	–0.253	0.011
Soluble N	g/kg N	22	757	–0.489	0.219

WSC, water soluble carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber.

Table 3. Effect of protein level on pH, organic acids and ethanol concentration of silage.

Parameter	Unit	n	Intercept	Slope	p-value
pH	no unit	119	3.85	0.0036	0.010
Lactate	g/kg DM	111	42.7	0.068	0.388
Acetate	g/kg DM	111	15.4	0.096	0.074
Propionate	g/kg DM	82	9.00	–0.022	0.567
Butyrate	g/kg DM	61	2.77	0.025	0.658
Formate	g/kg DM	25	4.93	0.016	0.494
Ethanol	g/kg DM	74	0	0.072	0.022

Table 4. Effect of protein level on digestibility, microbial population, nitrate and ammonia concentration of silage.

Parameter	Unit	n	Intercept	Slope	p-value
OMD	g/kg DM	17	274	1.99	<0.001
LAB	log cfu/g	52	6.32	0.018	0.025
Yeast	log cfu/g	73	16.0	–0.035	0.286
Mould	log cfu/g	52	7.23	0.0005	0.978
Nitrate	g/kg DM	12	0	0.125	0.028
NH_3	g/kg N	95	0	0.488	0.018

OMD, organic matter digestibility; LAB, lactic acid bacteria; NH_3, ammonia.

Higher ammonia concentration by increasing protein level in the ensiled material indicates an elevation on the extent of proteolysis. During ensiling, protein is degraded to amino acids and further fermented to α-keto acid and ammonia by the action of plant and microbial proteases [21]. Since ammonia is an alkali substance, it leads to an increase in the pH value of the silage. Furthermore, formation of ammonia means a solubilization of nitrogen and therefore could not be recovered in the dry matter, resulting an increase of DM loss. Such condition has a drawback consequence in which it reduces the efficiency of dietary nitrogen utilization by the livestock [22].

Protein apparently promotes the proliferation of LAB as indicated by the positive slope between protein level and LAB population, particularly the hetero-fermentative LAB. A tendency of increase of acetate concentration as well as an elevation of ethanol concentration in the present meta-analysis support our hypothesis. Higher OMD with increasing level of protein is expected since generally the compound is easily digested in the digestive tract of various animal species including ruminants [23]. However, certain protein fractions are hardly digested such as neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP), but they present in feeds in relatively small proportions. These fractions had been shown to have negative effects on crude protein digestibility in the rumen [24].

4. Conclusion
It can be concluded that higher protein level induces proteolysis in the silage and reduces its fermentative quality.

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