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Issues with Tropical and Temperate Ensilage Protein and Amino Acid Feeds Utilization: A Research Note

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Abstract

Alfalfa protein breakdown was to soluble NPN of oligopeptide-N, AA-N, amide-N, amine-N and NH3-N. Acidity (pH) and moisture (Aw) are critical in determining extent of fermentation and changes in composition. Further changes in digestive flows and post-prandial plasma AA are indicators of protein status. Dual-purpose cropping and tree plant cropping was with ensiling management of the undergrowth. On-farm field-drying and probiotic additives are promising. It is suggested acidity with propionic acid and microbial inoculants together with field-drying and chop length are required to optimize profile qualities in silage. It is proposed use of denaturing with acid and dust cropping with a hypothetical PNA-Auxin repressor to plant protease. Further study with field-drying to follow is needed. Feeding HIS, ARG and LEU AA supplement to change GRH and GH profiles could be used to promote LBM in production. Dual-purpose cropping can expand subsistence to mixed farming with expanded livestock products and services and resources. PNA-Auxin and PNA-ARF penetrates the plant shoot tips to deliver a TF mRNA to boost proteins in residual cell tissues. Ensiled % AA-N delivery per os to per duodenum was higher; yet total AA-N flow was higher in the control. It is suggested that "bulk" flow was less but with a "tighter" conversion on TAA. FAA was 145% higher in the ensiled versus the fresh control indicating the ENU with less PFAA supplied. FAA on the ensiled diet is high inferred to be more soluble and escape lower from the rumen. WSC are less supplied in fermented forage with VFA being lower and presenting the question whether WSC should be supplied for energy and also with EFE through breaking down of polymers of lignocellulose. It was surmised, although not known, that higher dilution rate (% hr^{-1}) was true on the fresh diet compared to the ensiled although end-products may initially detract with feed but that further digestion in the fresh feed may be higher with intake. Plasma AA before and after absorption or feeding are indicators of synthesis and breakdown. No data was available on N status; protein nutrition on neat silage was probably due to net efflux of AA with mobilization before influx with feeding and subsequent insulin action for uptake. Estuarine aquatic plant spp., water hyacinth used in the Philippines and duckweed studies in Australia, and post-harvest treatment with chemical additives and anti-microbial agents to help control potential transfer of diseases. "Greens" as supplements has yet to be established for anti-microbial properties for animal health and welfare. In conclusion, alfalfa silage fed at standard 0.6 cm particle size and wilted led to dramatic changes with AA breakdown, dramatic changes in duodenal AA flows from escape and recapture into microbial cells. Also N status of animals was compromised by lack of adequate "stores", mobilized, resulting in a net decrease in total plasma AA with insulin-dependent uptake to tissue.

Keywords

Silage, Protein Degradation, Duodenal Amino Acid Flows, Plasma Amino Acids, Protein Utilization

1. Introduction

This paper outlines the considerable breakdown of alfalfa silage protein nitrogen (N), to more soluble non-protein N (NPN), to oligopeptide-N and amino acids-N, with amide-N, amine-N and NH3-N, as the other major NPN components. In the particular study of [1] (**Table 1**), the total amino acids (TAA) were documented for precision-cut (0.6 cm) and wilted alfalfa silage to bring about a −9.6% change in TAA, and however, did not indicate necessarily the solubility of protein in the ensiled material due both to oligopeptide-N and free amino acid-N (FAA-N). Of interest are what these changes pose to digestion and post-absorptive metabolism with the need to elucidate the principles for tropical and temperate settings, using the observation that acidity (ideally a pH low = 3.8) and moisture levels or water activity (Aw) are critical parameters in determining the extent of fermentation and the compositional changes in silage. Further outlined are dramatic changes of digestive flows to the small intestines, viz the duodenum, of total essential and non-essential amino acids (EAA, NEAA). Post-prandial plasma amino acid levels 3 hrs. after feeding, a metabolic indicator of protein status together with proposed whole body animal measurements, are discussed.

2. Approaches to Boosting Cropping of Ensilage Protein

The use of technological approaches to further increase protein content of silages would be a considerable socio-cultural advance for the small farmer in developing world settings and has already been mentioned in the case of dual-purposed food-feed cultivars for crops in Australia with protein introduced genetically by recombination for increased "storage" in forage for utilization.

Table 1. Compositional changes due to ensilage and digesta flows from the rumen to the duodenum and post-absorptive changes in plasma with sheep fed neat alfalfa as forage.

Parameter	Treatment	
	Fresh (frozen) Alfalfa Control	Alfalfa Silage
Feed Amino Acids Composition (%)		
Total	60.25	53.96
Essential	34.23	27.53
Non-Essential	26.02	26.43
Feed Digesta Flows to the Duodenum, (g/d)		
Total	139.34a	109.49b
Essential	73.25a	57.38b
Non-Essential	66.09a	52.11b
Post-prandial Differences in Plasma Amino Acids (nmol/mL)		
Essential	−575a	−106b
Non-Essential	−320	71 NS

Taken from: [1] [6]. a, b: Means bearing different letters are statistically different ($P < 0.05$) N = 8. NS = not significant ($P > 0.05$).

Also here, grazed forages using larger land holdings, typical in Asia, for e.g., tree crops in commercial plantations (e.g. oil palm and coconut trees) with animal grazing, for e.g. by cattle, sheep, and goats for the undergrowth presenting a "plus-plus" situation between plantation and animal farming (see **Figure 1**), with the promise of improving grazing the undergrowth and their stocking rates together with boosting carbon sinking (C-sinking) and forage protein nitrogen (protein-N) for feeding. This would lead to the new development of harvest management of the undergrowth year round using ensilage as a technology. Although not expanded upon here this could entail attendant postharvest treatment methods or techniques that can treat fibre for greater digestibility, treat protein-N to boost its utilization and supplement with additional NPN like urea-N and NH3-N.

The case of using wilting or field drying on-farm together with microbial inocula as prebiotic additives is to be applied on ensilage is promising as discussed following.

2.1. Practices of Wilting Crops with Chemical and Biological Additives

A fine-particle size or chop length is requisite for anaerobiosis in the ensiling biomass, at 0.6cm, to attain acceptable profile nutritive qualities with respect to ensiling conditions. It was observed that increased dry matter (DM) content reduced proteolysis and formation of amino acids during ensilage [2]. It has been

Livestock		Tree Crops
1. animal feed supplements (energy, protein, fibre) seeds, hulls etc.	<--------------agri-biproducts <---------------------------	fruits, stalks
2. grazing, manure	--->	less herbicides, weeding
	----------->undergrowth fertilizer ------------------------>	tree fertilizer
3. animal feed	<--------------- fertilizer to pasture <-----------------------	seeds effluent
4. animal feed	<---	legumes/shrubs broadleaf shrubs
5. animal toxicity	<---	browsed fruits
6. animal browsing	-->	damage to young tree crop foliage

Figure 1. The mixed-farming model with tree cropping plantation and small ruminant production. Taken from [11].

noted that addition of formic acid can increase peptide concentration in alfalfa silage and even their size [3], indicating their decrease in degradation from protein with ensilage. Prebiotic inocula from Lactobacilli and Pediococcus were found to reduce proteolysis especially in silage of higher dry matter content of 23% versus 28% [4]. It is suggested that optimum profile qualities in ensilage requires an acidity with, for e.g. formic acid addition or commercial microbial inocula and at a DM content with wilting and perscribed chop length.

2.2. A Low-Protease Cropping Approach: Use of Enzymes or PNA-Auxin Regulatory Inhibitors

There are certain enzymes that are expressed on cell death [5], e.g. with harvest of plant forage crops, that play an ecological role to eventually breakdown plant protein and if left to eventually compost, will return back to the topsoil for its fertility. There are various approaches here to inhibit protease activity when expressed in dying plant tissue including approaches to denature proteinaceous enzymes with dilute acid if available as an agricultural additive, and more specific and involved, the use of a hypothetical peptide nucleic acid (PNA)-auxin regulatory biologic that can be dust cropped, during harvest. Field-drying or wilting conditions follows and the issue of its use with the measures mentioned previous, will need further investigation [5]. It is unlikely at this time to apply competitive or allosteric inhibitors to plant proteases during harvest of biomass prior to silo filling as they may not be feed grade for animals and for the consumer.

2.3. A Proposal to Formulate New Technology for Escape Protein with Forage

Processing ensilage proposed with pelleting dried forage as escape protein supplements including supplementation with functional amino acids such as histidine (HIS), arginine (ARG) and leucine (LEU) are known to change growth releasing hormone (GRH) and growth hormone (GH) profiles and could be used as non-steroidal growth promoters of lean body mass (LBM) for animal production. The use of a combination of organically produced live yeast culture (LYC) and dead yeast culture (DYC) as the bulk should be further developed to be used for feeding.

2.4. Ensilage, Dual-Purpose Cultivars and Use of Proposed PNA-Auxin Technology for Plant Crops

Non-GMO, dual-purpose cultivar cropping using the proposed genetic regulatory organismal (GRO) approach can transform feed supply to expand on livestock resource planning, transforming availability from mixed farming, moving away from more basic subsistence settings, allowing breeder stock into the picture, livestock ownership for trading or sale of animal products from slaughtered meat or dairy production. There is also the matter of capital from animal stock which can be converted to cash flow and financial assets. Nutritive value (NV) can be improved due to modifying composition of more common protein components in plant cells that are functional as "stored".

Due to biosafety and regulatory concerns for GMO-based technology, non-GMO approaches present new possibilities. Here PNA for delivery into the cell via an auxin or auxin-like regulatory factors (ARF) covalently attached in the PNA-auxin complex penetrates the plant's tissues at its shoot tips to deliver carriered conjugate transcription factor (TF) mRNA to boost expression of the most abundant proteins in plant cells of residual tissues to create the dual-purposed modeled plants.

Transcription factors (TF) are used with their target genes as to their binding promoter (P) regions for decreasing, in this case, called TF "silencers", or increasing, in this case, called TF "enhancers", for the expression of these proposed markered or targeted genes further "downstream" on the forage plant's DNA. Research establishing biosafety is still down the road to be in place for regulatory reasons including environmental and human safety concerns.

3. Inferences from the Study of the Conversion from per Os to Duodenal Flows with Rumen Microbial Cell Protein (MCP) versus Escape Protein

3.1. Protein Delivery per Os to per Duodenum on the Ensiled versus the Fresh Forage Diet

The ensiled diet has a higher % delivery of AA-N per os to ruminal outflows at the duodenum [1] and yet the total flow of AA-N was higher on the fresh control. It is hypothesized that for a given flow on the restricted diet fed to metabolic body size (BW.75 kg) the "bulk" was less on the ensiled versus fresh diet despite the "tighter" conversion proportionately on total amino acids between per os and per duodenum.

It was not known in the study for [1] whether the efficiency of nitrogen uptake (ENU), indicating the capture of PFAA-N, although as indicated by [7], ensiled versus the non-ensiled diet has less capture of PFAA-N and thus contributes less support to the efficiency of MCP synthesis and flows to the duodenum compared to the control diet.

In the study of [1] the proportion of free amino acids (FAA) of the total was 145% higher with the ensiled diet than the fresh (frozen) control suggesting that the protein was more soluble for alfalfa silage which would indicate the ENU with less pre-formed amino acids (PFAA) likely supplied on the ensiled diet vs the fresh diet. It was mentioned in [8] that the FAA in rumen fluid are very low indicating they are highly degraded.

In the study of [1], the FAA being proportionately higher on the alfalfa silage diet, would make it more soluble and would indicate that escape from the rumen was proportionately lower, as inferred.

3.2. The Role of Energy Supply Contributing to MCP Synthesis on the Ensiled versus the Fresh Diet

The author infers that sugars and other water-soluble carbohydrates (WSC) are in less supply or availability in the fermented forage feed as indicated in the case of [1], with the rumen volatile fatty acid (VFA) concentrations being lower for the silage in that study, and presents questions whether WSC in general or certain sugars be supplied for energy as carbohydrates with forage or as can also be done via application of exogenous fibrolytic enzymes (EFE) [9] that can lead to greater microbial digestion in the rumen through breaking down polymers of lignocellulose and hemicellulose such as with lignases, like ferulic acid esterases (Fae), etherases, cellulases and hemicellulases.

3.3. Dilution Rate in the Rumen with the Ensiled Diet versus the Fresh Control

It was not known whether the dilution rate based on controlled intake levels pegged to metabolic body size (BW.75 kg), and thus gut fill, also controlled by the greater solute concentration on the silage diet due to fermentation endproducts may with feed initially detract from a higher induced dilution rate (% hr^{-1}) on the fresh diet compared to the ensiled, but following further digestion to the total of endproducts of rumen fermentation inferred to be higher on the higher intake or fresh diet could have factored to a net positive effect for the fresh diet, although this remains undetermined in the study.

4. Post-Prandial Plasma Amino Acids as Metabolic Indicators of Protein Status

Plasma AA before and 3 hrs. after feeding with digestive absorption are the indicators of protein turnover as a whole, viz. for synthesis and breakdown, examples being end products of skeletal muscle tissue breakdown and certain amino acid-N end products. Other indicators of interest would be hormonal plasma levels, e.g. hGH and hGRH and in general the plasma LMW-proteome for other possible biomarkers not discussed here.

In the study of Flores *et al.* [6] measurements of N status or balance were not available [10] although in principle they were fed above maintenance corrected for metabolic body size (BW.75 kg) (intakes of 83.1a vs 67.6b, g/d - kg 0.75; a, b $P < 0.05$) which would have made the discussion of plasma changes before and after feeding more straightforward due to the hypothesis here that in the case of the level of protein nutrition used with neat alfalfa silage consumption (without concentrate as supplement) given our data on plasma amino acid levels (see: **Table 1**), this was probably due to a net efflux of amino acids with mobilization from tissues as a pre-prandial "background" before the influx of amino acids from feeding into plasma and their subsequent decrease due to insulin secretion and action for uptake to tissues. The transaction of amino acids between tissues is in part due to gluconeogenesis in the liver as with fasted animals which mobilize it for glucose to support energy for the brain.

Another way to assuage from using a large animal research unit (LARU) with whole body measurements, not available at the time at the Macdonald Campus farm facility at McGill University, Qc Canada, is to determine the heats of maintenance and growth and body composition such as the lean body mass (LBM) and total body fat than just from differences with N balance alone.

5. Controlling Potential Zoonoses from Tropical Aquatic Plants

With respect to estuarine aquatic plants, e.g. water hyacinth, utilizable as feed ensilage (e.g. used already in the Philippine Islands) and duckweed (already studied in Australia) and their post-harvest treatment using chemical additives such as with formic/propionic acids and anti-microbial chemical additions. Apparently, factors in the environment like climate change, e.g. global warming, animal density, *i.e.* more intensive farming practices, and population growth, are implicated in the incidence of transferring diseases, e.g., as in the case of COVID-19/ variants originally in chicken livestock to humans creating havoc in the daily lives of peoples world-wide.

Recently brought to the attention of scientists is the natural practice of road-side, bunds or yard feeding of green grasses and weeds with teathered pigs, chickens, goats and other cattle leading to the author's speculation of using "greens" as supplements perhaps in dehydrated and/or pelleted form. Although it has not been established what anti-microbial properties and their agents (for e.g. long fibres, phytochemicals) are responsible for maintaining or improving animal health and welfare.

6. Conclusion

It has been concluded with our fed alfalfa silage, used at a precision-cut standard 0.6 cm particle size and also wilted, led to dramatic changes with AA breakdown with processing, that is, the total and non-protein amino acids as indicated from the free amino acids (FAA), but not including the presence of oligopeptide amino acids with degradation in protein-N to oligopeptide-N (both inferred) and the FAA-N with the latter's further loss to amine-N, amide-N and, largely, NH3-N. There were dramatic changes in duodenal AA flows from escape protein, which would depend on protein solubility in the rumen and its breakdown followed by the recapture into microbial cells for the MCP flowing from the rumen stomachs of sheep. Finally, it can be inferred that status of animals was compromised by lack of adequate protein "stores" preprandially in mobilized amino acids from protein in plasma, for e.g., the muscle and liver, and resulting in a net decrease in total plasma amino acids, including EAA after feeding rather than the net increase expected with its influx 3 hrs. postprandially after per os inputs and following flow after the rumen stomach to the duodenum together with their insulin-dependent uptake into tissues like skeletal muscle and liver.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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