



## Original Research

# Protein and amino acid digestion characteristics of two forms of preserved lucerne forage fed to mature horses

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### Summary

A trial was conducted, using 12 mature thoroughbred horses in a cross-over design, to compare the protein and amino acid digestibility of dry lucerne chaff (LC) against a controlled fermented lucerne (CFL) product (HNF Fiber<sup>®</sup>; Fiber Fresh Feeds Ltd, Reporoa, New Zealand). Crude protein levels were higher in CFL compared to LC, and when individual amino acids were analysed, the majority (88%) of these were also higher. Crude protein digestibility was significantly (18%) higher in CFL diets ( $P < 0.001$ ) compared to LC. Significant differences ( $P < 0.05$ ) were seen in individual amino acid digestibility in favour of the CFL diet for lysine (24%) and methionine (30%), as well as threonine, histidine, tyrosine, leucine, isoleucine, valine, alanine, proline, serine and aspartamine. Although amino acid requirements in horses are still poorly defined, the higher availability of amino acids in CFL compared to LC should be taken into account when formulating horse diets including these feedstuffs.

**Keywords:** Lucerne: protein: processing: horses: digestion

(Received 12 November 2012 – Accepted 1 February 2013)

### Introduction

Lucerne (*Medicago sativa*), also known as alfalfa, is a legume forage commonly fed to horses. Previous studies have indicated that the preservation method used on lucerne impacts its energy availability and digestible energy characteristics in horses (Waldron *et al.*, 2012). Lucerne hay has been found to have higher dry matter and protein digestibility, and enhanced mineral absorption compared to other forages (Crozier *et al.*, 1997). It is common practise to use lucerne as a source of protein and lysine in commercial horse feeds, as it is well recognised for its high levels of these nutrients. In other species, it is known that the preservation process used with forages, e.g. drying or fermenting/ensiling, can impact on not only nutrient level but also digestibility. Horses have been shown to retain lucerne longer than oat straw in their gut, which can increase the overall

diet digestibility, when fed alongside other feedstuffs including grains (Cuddeford *et al.*, 1995). When fed either pelleted or hay forms of lucerne, trial data from horses has shown that processing affects feed intake, with pelleted lucerne providing less digestible fibre, requiring higher daily intake (Haenlein *et al.*, 1966). The following trial was conducted as part of a previous trial (where feed and faecal samples had been collected), which examined the differences in energy digestion between CFL and dry lucerne chaff (LC). In the current trial, the protein and amino acid digestibility in CFL and LC was compared to examine whether processing had an impact on the availability of these nutrients.

### Materials and Methods

Twelve non-racing Thoroughbred horses, seven mares and five geldings, ranging in size from 15 to 16.1

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hands (mean  $\pm$  SE,  $15.6 \pm 0.13$  hands) and an average body condition score of 4 (on a 1-9 scale) were housed in  $3.6 \times 4$  m pens, with a bedding of wood chips. The trial commenced with a 7-day adaptation period, where dry LC (8.24 kg per day split into two feeds) was fed alongside decreasing amounts of a complete and balanced commercial feed (Dunstan Coolfeed; Dunstan Nutrition Ltd, Hamilton, New Zealand) split into two feeds (3.00 kg on days one to three, 2.25 kg on days four and five, 1.50 kg on day six and 0.75 kg on day seven) and supplied to each horse. This was to ensure that all horses were on the same nutritional regime before the trial diets were introduced, to limit any effects of previous diet, and to gradually introduce lucerne into their daily ration. On day eight, horses were randomly allocated to one of the treatment forages; either the CFL (HNF Fiber<sup>®</sup>; Fiber Fresh Feeds Ltd., Reporoa, New Zealand) or LC (sourced from a single batch harvested from one site in Wairarapa, New Zealand) for two further 7-day periods, in a cross-over design, to give 12 replicates per diet.

Horses were supplied with 18.8 kg CFL, or 8.24 kg dry LC per day, split into two feeds, given at 06:00 and 18:00. As there were no specific DE or dry matter (DM) figures available at that time for controlled fermented lucerne, the intake calculations were based on standard NRC values of 9.6 MJ/kg lucerne hay (91% DM) for LC and 9.7 MJ/kg fresh lucerne (full bloom; 40% DM) for CFL, to provide 72 MJ per day energy intake (NRC, 1989; 2007). Feed refusals were weighed and recorded on a daily basis. Daily faecal output was measured for each horse for twelve hours on the last day of each 7-day period and sub-samples of feed and faeces were taken from each horse for protein and amino acid analysis. Samples were analysed for DM using a convection oven at 105°C (AOAC, 2005; methods 930.15 and 925.10), crude protein (by LECO total combustion method (AOAC, 2005; method 968.06) and amino acids (Hydrochloric acid hydrolysis followed by HPLC separation, AOAC, 2005; method 994.12). Digestibility was calculated from feed intake and levels of protein and individual amino acids from feed and faecal samples (DM basis).

Data were analysed by the General Linear Model procedure of Unistat 5.5 (Unistat UK Limited), with the cross-over designated as a time replicate (Unistat, 2007).

## Results and Discussion

The analysed levels of crude protein and amino acids in CFL and LC are shown in Table 1. Horses fed CFL had

**Table 1.** Protein and amino acid levels (%) in controlled fermented lucerne (CFL) or lucerne chaff (LC) (dry matter basis)

Nutrient	LC	CFL
<b>Crude protein</b>	15.1	21.7
<b>Aspartamine</b>	1.84	3.21
<b>Threonine</b>	0.60	0.71
<b>Serine</b>	0.59	0.76
<b>Glutamic acid</b>	1.33	1.34
<b>Proline</b>	1.16	2.32
<b>Glycine</b>	0.69	0.77
<b>Alanine</b>	0.64	0.81
<b>Valine</b>	0.84	1.06
<b>Isoleucine</b>	0.64	0.80
<b>Leucine</b>	1.00	1.18
<b>Tyrosine</b>	0.49	0.58
<b>Phenylalanine</b>	0.68	0.79
<b>Histidine</b>	0.30	0.45
<b>Lysine</b>	0.90	0.90
<b>Arginine</b>	0.62	0.48
<b>Cysteine</b>	0.22	0.24
<b>Methionine</b>	0.21	0.27

higher ( $P < 0.0001$ ) crude protein intakes, compared to those fed LC due to its higher protein level (21.7% versus 15.1% DM basis), whereby protein intake was 191 g/d and 109 g/d for CFL and LC respectively. Levels of the amino acids aspartamine and proline were markedly higher in the CFL compared to LC, illustrating how processing methods can affect amino acid levels in the same plant material.

Protein and amino acid digestibility was calculated for each horse, based on individual feed intakes. The results are shown in Table 2. Significant increases ( $P < 0.05$ ) in the digestibility of protein, lysine, methionine, threonine, histidine, tyrosine, leucine, isoleucine, valine, alanine, proline, serine and aspartamine were observed (Table 2) for CFL compared with LC. Increases in both the level of protein and amino acids in the diet and quality of these in the horses diet result in increased absorption of amino acids to contribute to the amino acid pool for tissue synthesis and repair (NRC, 2007). Higher digestibility is suggestive of more efficient use of protein (irrespective of level of intake) and higher availability of amino acids to the horse, and less waste in manure output of nitrogenous breakdown products.

The main limiting amino acids, for diet formulation purposes, are lysine, methionine and cysteine, and absolute levels (on a dry matter basis) for these amino acids were similar for both forms of lucerne (Table 1). However, the digestibility of lysine (24%) and methionine (30%) was significantly higher for CFL compared with LC ( $P < 0.05$ ). These findings demonstrate that the method of processing affected the availability of

**Table 2.** Protein and amino acid digestibility (%) of controlled-fermented lucerne or dry lucerne chaff in non-racing Thoroughbred horses (n = 12)

	LC	CFL	Difference, %	P value
Crude protein	64.3 <sup>a</sup>	76.1 <sup>b</sup>	18	0.0008
Lysine	62.1 <sup>a</sup>	76.9 <sup>b</sup>	24	0.0001
Methionine	49.6 <sup>a</sup>	64.7 <sup>b</sup>	30	0.0021
Cysteine	57.4	54.9	NS	0.5347
Threonine	63.8 <sup>a</sup>	71.8 <sup>b</sup>	13	0.0189
Arginine	75.3	74.8	NS	0.7997
Histidine	56.3 <sup>a</sup>	71.3 <sup>b</sup>	27	0.0006
Phenylalanine	64.7	70.9	10	0.0547†
Tyrosine	58.7 <sup>a</sup>	72.7 <sup>b</sup>	24	0.0006
Leucine	67.5 <sup>a</sup>	73.7 <sup>b</sup>	9	0.0424
Isoleucine	62.8 <sup>a</sup>	71.8 <sup>b</sup>	14	0.0096
Valine	64.9 <sup>a</sup>	74.7 <sup>b</sup>	15	0.0034
Alanine	64.1 <sup>a</sup>	73.8 <sup>b</sup>	15	0.0044
Glycine	59.6	64.8	9	0.1589
Proline	79.1 <sup>a</sup>	90.9 <sup>b</sup>	15	<0.0001
Glutamic acid	67.9	70.9	NS	0.3134
Serine	65.7 <sup>a</sup>	75.1 <sup>b</sup>	14	0.0042
Aspartamine	75.0 <sup>a</sup>	87.0 <sup>b</sup>	16	<0.0001

Means in a row not sharing a superscript differ significantly ( $P < 0.05$ ).  
NS = not significant; † indicates strong trend in data.

these amino acids to the horse. Protein quality and digestibility is a major consideration in animal feeds, including horse feeds, as it forms the basis of hoof, hair, muscles, organs, tissues and immunity. It helps to explain why improvements in top-line and muscling are typically observed on horses fed the CFL product (Waldron *et al.*, 2012).

### Conclusions

These findings demonstrated that the levels and digestibility of protein and its constituent amino acids in CFL were, in the majority of cases, significantly higher compared to LC. This indicated that the level of available amino acids was generally higher in the CFL compared with LC.

Data regarding protein and amino acids requirements in horses are limited compared to other species, relying on extrapolation and limited published papers. Further work into actual protein and amino acid requirements of horses is required, which can then be considered in relation to the use of such products as CFL. CFL may be useful in building muscle and top-line, especially in growing horses or for breeding animals (which have the highest protein requirements). It may also help in competition horses or those in hard work, whereby more protein is required to meet increased muscle turnover demands. This would certainly be the case for heavy weight breeds, such as Warmbloods, which are more heavily muscled in comparison to Thoroughbreds.

### Acknowledgements

This research was funded by Fiber Fresh Feeds Ltd, Reporoa, New Zealand.

### Declaration of interest

I.D. Pryor and N.L. Stowers are employees of Fiber Fresh Feeds Ltd, Reporoa, New Zealand.

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