The effect of dietary protein on reproduction in the mare. I. The composition and evaluation of the digestibility of dietary protein from different sources

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ABSTRACT

Four rations that differed in their crude protein and essential amino-acid content were compiled. Digestibility of the crude protein and essential amino-acid contents were determined biologically in a feeding trial using 4 Anglo-Arab stallions. Their respective daily diets were: Diet 1: 2 kg cubes, 5 kg tef hay *(Eragrostis tef)*; Diet 2: 2 kg cubes, 5 kg lucerne hay *(Medicago sativa)*; Diet 3: 2 kg cubes, 5 kg tef hay, 200 g fishmeal; Diet 4: 2 kg cubes, 5 kg lucerne hay, 200 g fishmeal. The concentrations of the amino-acids threonine, iso-leucine, leucine and arginine were increased in the total ration when lucerne hay replaced the tef hay while fishmeal supplementation increased the methionine and lysine contents, which provided a wide range of concentrations of digestible amino-acids in each of the 4 rations.

Key words: amino-acids, equine, nutrition.

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INTRODUCTION

The foaling percentage of registered Thoroughbred mares in South Africa was 57 % in 1981/82 and 60 % in the 1990/91 seasons[4], which indicates that there has been very little improvement in the fertility of mares during the last decade in spite of improved stud management and veterinary supervision. Very little research has been carried out in the past decade on the effect of nutrition on reproduction in the Thoroughbred mare. As early as 1965 preliminary work revealed the possible advantageous effect of good nutrition in preventing early embryonic loss and also the shortening of the transitional period of mares[16,17]. These studies suggested that the protein content of the diet in terms of quantity and quality may play an important role in the fertility of mares[16,17].

The existing nutritional guidelines and recommendations for horses (National Research Council (NRC), Washington DC)[8], are based partly on research carried out on non-reproducing horses, mainly of the pony types, and also extrapolation from research in ruminants. Anatomically and physiologically the horse can be regarded as a simple-stomached animal with no microbial fermentation in the stomach[11,12,13]. In contrast, the ruminant has a well-developed 4-stomach system where the rumen acts as reservoir for microbial activity, resulting in the production of highly digestible microbial protein[12,14]. In the Equidae, protein digestion commences in the stomach and 60–70 % of the digestion and absorption is completed in the small intestine before reaching the caecum[2,6,7,9]. The fact that no microbial protein is produced in the stomach of the horse accentuates the need for an adequate supply of easily digestible protein containing an adequate quantity of all the essential amino-acids in the diet. While the horse cannot produce its own essential amino-acids by the synthesis of microbial protein to the same extent as ruminants, it is uncertain to what extent Equidae can utilise caecally-produced microbial protein [2,10].

Utilisation of non-protein nitrogen (NPN) by the horse is also controversial. In Equidae, NPN can only be utilised by bacteria in the caecum. A very small percentage of NPN reaches the caecum, as urea is usually broken down by the enzyme urease and absorbed as ammonia in the small intestine. In the liver, the ammonia is converted to urea and excreted in the urine and milk[2]. Furthermore it has been suggested that the end-product of the proteolytic process in the caecum is ammonia rather than an increase in free amino-acids[10].

Evaluation of the diverse opinions in the literature shows that horses, in contrast to ruminants, require high-quality protein in their diet. Consequently, this series of 7 articles investigates the specific effects of the quality (amino-acid content) and quantity of dietary protein on certain aspects of the reproductive cycle of the mare. This includes the anovulatory period, the transitional period, the ovulatory period (breeding season), pregnancy, *post partum* period and lactation and the growth of the suckling foal. In order to conduct studies of this nature it is necessary to determine how protein from different raw materials is utilised.

The purpose of the present study was to evaluate the apparent digestibility of certain essential amino-acids in 4 different diets. These diets are also used in the subsequent studies, where the effects of dietary protein on reproduction are discussed.

MATERIALS AND METHODS

Four Anglo-Arab stallions aged 5–8 years and weighing between 440 and 510 kg were used in this study.

Four diets, as shown in Table 1, were evaluated. Diet 1, also referred to as the control diet, consisted of tef hay (*Eragrostis tef*), which is generally used as a source of roughage for horses in most parts of South Africa. This ration was supplemented with cubes (Table 2) not containing any fishmeal. In Diet 2 the tef hay was replaced by lucerne (*Medicago sativa*), a high-quality legume hay. Diets 3 and 4 had the same composition as Diets 1 and 2 respectively but were each supplemented with 200 g of fishmeal.

The 4 stallions were used to evaluate the diets on a 4 × 4 latin square procedure[5]. The stallions were kept in cement-floored stables without bedding. After an adaptation period of 7 days for every diet, the total faecal excretion per 24 h period for every diet was collected daily for 7 days. After the faeces were weighed, 300 g samples were collected and dried in an oven for 6 h at 70 °C to obtain the dry-matter content. These samples were then milled in a stainless steel mill through a

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1 mm screen and stored for further analysis.

The fishmeal, mixed with the cubes, was fed at 09:00 and the hay at 11:00. Clean drinking water was freely available.

The crude protein content of each of the 4 diets was determined by the macro Kjeldhal method[1]. Total digestible nutrients (TDN) were determined using the *in vitro* technique described by Engels and Van der Merwe[3]. The amino-acid content was determined after acid hydrolysis of each sample in a sealed glass tube using a Beckman (Model 6300) automatic amino-acid analyser.

Statistical analysis

The results were analysed as a 4×4 latin square[5]. The basic design of the experiment can, however, be regarded as a 2×2 factorial. It is acknowledged that a latin square design does not allow for the assessment of interactions but it was impossible to conduct the digestibility study in any other way. In the presence of significant *F*-values for treatment in the analyses of variance table, protected least significant differences were calculated for the comparison of means.

RESULTS

The crude protein (CP) as well as the concentration of certain of the essential amino-acids of the total daily diets are given in Table 3.

The apparent crude protein (CP) and amino-acid digestibility of the 4 rations were determined by comparing daily intake and faecal excretion of the different components as indicated in Table 4. Estimation of the apparent digestibility of the CP and amino-acids was based only on the results obtained from faecal samples and did not include urine samples.

The supplementation with fishmeal increased the digestible threonine, methionine, iso-leucine and lysine content of the rations, although it was not significant in all cases. Significantly increased levels of digestible threonine, iso-leucine, leucine, lysine and arginine contents were found in the diets containing lucerne hay compared with the diets containing tef hay. These differences are reflected in the apparent digestibility of these amino-acids (Table 4).

DISCUSSION

In formulating the 4 diets, lucerne hay was considered to be a good-quality protein hay compared to tef hay, since the threonine, iso-leucine, leucine, lysine and arginine content of lucerne hay was nearly double that of tef hay, with the exception of methionine, which was

Table 1: **Composition of the four diets.**

Diet[a]	Cubes (kg)	Lucerne hay *(Medicago sativa)* (kg)	Tef hay *(Eragrostis tef)* (kg)	Fishmeal (kg)
1 (TC)	2		5	
2 (LC)	2	5		
3 (TCF)	2		5	0.2
4 (LCF)	2	5		0.2

[a]TC = tef hay and cubes; LC = lucerne hay and cubes; TCF = tef hay, cubes and fishmeal; LCF = lucerne hay, cubes and fishmeal.

Table 2: **Composition of the cubes.**

Raw material	%
Maize	10.0
Oats	12.0
Wheat	42.0
Sunflower oil cake	8.1
Cottonseed oil cake	2.5
Feedlime	2.5
Salt	1.6
Lucerne	11.0
Molasses	10.0
Vitamin mineral premix	0.3

Table 3: **Mean crude protein** (%) **and amino-acid content** (%) **of 4 diets as determined on daily dietary intake.**

Component	Diet[a] 1 %	2 %	3 %	4 %
Crude protein	10.8	14.0	12.06	15.15
Amino-acids:				
Threonine	0.30	0.50	0.35	0.53
Methionine	0.11	0.10	0.15	0.14
Iso-leucine	0.38	0.62	0.43	0.66
Leucine	0.71	1.04	0.68	1.00
Lysine	0.55	0.76	0.65	0.86
Arginine	0.62	0.98	0.59	0.94

[a]Diet 1 = tef hay and cubes (7 kg/day/horse); Diet 2 = lucerne hay and cubes (7 kg/day/horse); Diet 3 = tef hay, cubes and fishmeal (7.2 kg/day/horse); Diet 4 = lucerne hay, cubes and fishmeal (7.2 kg/day/horse).

Table 4: **A comparison of the percentage apparent digestible crude protein** (DCP) **and the percentage apparent digestible amino-acid content of 4 diets as based on the analysis of faecal samples**.

Component	Digestibility of diet[a] 1	2	3	4	S.E. (mean)	*p*	LSD[b]
DCP	8.8 a[c]	11.3 b	10.2 c	12.7 d	0.17	**	0.480
Amino-acids:							
Threonine	0.211 a	0.435 b	0.271 c	0.434 b	0.007	**	0.490
Methionine	0.087 a	0.077 a	0.130 b	0.188 b	0.002	**	0.012
Iso-leucine	0.284 a	0.510 b	0.334 c	0.545 b	0.007	**	0.046
Leucine	0.566 a	0.870 b	0.543 a	0.835 b	0.009	**	0.061
Lysine	0.412 a	0.599 b	0.521 c	0.703 d	0.008	**	0.059
Arginine	0.547 a	0.899 b	0.521 a	0.864 c	0.004	**	0.029

[a]Diet 1 = tef hay and cubes; Diet 2 = lucerne hay and cubes; Diet 3 = tef hay, cubes and fishmeal; Diet 4 = lucerne hay, cubes and fishmeal.
[b]LSD = least significant difference.
[c]a,b,c,d = values in the same row followed by different letters differ significantly ($p < 0.01$).

practically identical in both roughage sources. As shown in Tables 1 and 3, it was apparent that a stepwise increase in essential amino-acid content was achieved with this dietary composition.

The CP consumption of the horses fed the 4 different diets varied between 756 and 1106 g/d (Diet 1 – 756 g; Diet 2 – 980 g; Diet 3 – 880 g; Diet 4 – 1106 g). According to the NRC guidelines the dietary CP requirements for maintenance of an adult horse of 500 kg is 656 g/day[8]. The only essential amino-acid specified by the NRC is lysine which is given as 23 g/day for a 500 kg horse[8]. According to the calculation of daily lysine intake of the horses (Table 3) fed the 4 diets, the animal on Diet 1 consumed 38.5 g/d; Diet 2 – 53.2 g/d; Diet 3 – 47.5 g/d and Diet 4 – 62.8 g/d of lysine, all of which more than meet these requirements.

As the purpose of this study, to be reported in subsequent articles, was to investigate the effect of dietary protein quality on reproduction, it was important that the energy content of the 4 diets should be as similar as possible. By estimating the energy content using the equation DE (MJ/kg) = 0.165 TDN (%) + 0.72[15], the digestible energy content of each diet was 10.75 MJ/kg (Diet 1), 10.95 MJ/kg (Diet 2), 10.66 MJ/kg (Diet 3) and 10.66 MJ/kg (Diet 4). The NRC guideline[8] for the dietary energy content for maintenance of a 500 kg horse is given as 10.1 MJ/kg. Therefore all 4 diets met the minimum energy requirements for a 500 kg horse for maintenance, and in addition the energy content in all 4 diets was similar.

According to the results none of the 4 diets were deficient in amino-acids or energy. Although this might be applicable to non-reproducing adult horses, it might not hold for brood mares, where increased demands for these components would be expected. Should any deficiencies be encountered in later studies in this series, these might be expected to involve the essential amino-acids threonine, leucine and/or lysine (Tables 3, 4), as the *in vivo* synthesis of certain essential amino-acids from other non-essential amino-acids in the horse is currently unknown.

CONCLUSION

According to NRC (1989) standards, the basic ration consisting of tef hay and cubes (Diet 1) met the protein and apparently also the essential amino-acid requirements of the non-reproducing adult horse. However, by replacing the tef hay with lucerne hay, the total amino-acid, the essential amino-acids and the protein contents exceeded those in the diet containing tef hay supplemented with 200 g of fishmeal. The results show that it is possible that a deficiency of threonine, iso-leucine and lysine could occur when the control diet (Diet 1) is fed to mares in late pregnancy and lactation, when there is an increased demand for these components. It is therefore recommended that when tef hay is the only source of roughage for reproducing mares, the diet should be supplemented with high-quality protein.

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