UNIVERSITY OF ALBERTA

PROTEIN UTILIZATION OF FORAGE-BASED DIETS BY HORSES

BY

ROBERT J. COLEMAN ©

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **PROTEIN UTILIZATION IN FORAGE-BASED DIETS BY HORSES** submitted by **ROBERT JOHN COLEMAN** in partial fulfillment of the requirements of the degree of **DOCTOR OF PHILOSOPHY** in **ANIMAL SCIENCE.**

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ABSTRACT

Three experiments were conducted in which the protein nutrition of the horse was examined. In the first experiment 104 extensively managed creep-fed foals exhibited gains that were 10% greater (P<.05) than those of non creep-fed foals but no differences were detected between 13 or 17% crude protein (CP) creep rations. The creep-fed foals also had a better (P<.001) general appearance, which had a positive (P<.0001) effect on sale price. In experiment 2, 60 weanling fillies fed diets with 60% cubed alfalfa and 40% concentrate containing 15, 17 and 19% CP gained .72, .70 and .71 kg/day (P>.05), respectively. In experiment 3, mature ponies cannulated in the distal ileum were used to determine partial and total tract digestibilities of an alfalfa cube diet (19% CP) and the three 60% forage 40% concentrate diets used in experiment 2. Total tract DM digestibilities were lower (P<.001) for the alfalfa cube diet than for diets containing 60% forage and 40% concentrate. There were no differences (P>.05) in prececal DM digestibility (34%-36%) for the 60% forage diets however the pre-cecal DM digestibility of the alfalfa cube diet (23.4%) was lower (P<.0001). Total tract CP digestibilities were 71.9, 73.4, 75.3, and 77.6% (P<.04) for the alfalfa cube, 15%, 17% and 19% CP diets, respectively. Corresponding pre-cecal CP apparent digestibilities were 47.9, 51.9, 55.6 and 60.0%

and, with the exception of the cube diet, there was an increase (P<.0001) in pre-cecal protein digestibility as dietary CP intake increased. Pre-cecal lysine digestibilities with the alfalfa cube, 15%, 17% and the 19% diets were 48.1, 51.1, 55.9 and 62.3% (P<.0001), respectively. True total tract, pre-cecal and post-ileal protein digestibilities for the 60% forage diets were 90, 89 and 72%, respectively. Comparable true lysine digestibilities were 97, 98 and 88%. Pre-cecal protein and lysine digestibilities of the alfalfa cubes were thus only 60% that of the concentrate mixture used in the 19% CP diet. In conclusion, although high quality alfalfa can contribute to the protein and lysine requirements of the horse, pre-cecal digesibilities are reduced when forages are fed. Consideration should therefore be given to intestinal availability of amino acids when formulating equine diets.

DEDICATION

I wish to dedicate this thesis to my family, my wife Julie and my children Stephen, Joanna and Elizabeth for all your support understanding and help during my Ph.D. program. Words can never express how much it was appreciated.

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CHAPTER 1

INTRODUCTION

The horse evolved as a grazing herbivore consuming small meals frequently. Forages still are a major source of nutrients for meeting the horse's daily requirements. However, the demands of today's horse industry have changed how horses are fed. The reliance on forage is reduced in favor of diets that are high in concentrates. Nonetheless the basis for feeding horses should be the optimal use of good quality forage, with other feeds added to supplement deficiencies.

The young growing foal is adequately nourished during early lactation by the milk produced by its dam (NRC 1989). However, by mid-lactation nutrients secreted in the milk may not be in sufficient quantities to support optimal growth of the foal (Gibbs et al. 1982). The use of supplemental feed is often recommended to supply additional nutrients to meet the needs of the growing foal prior to weaning. This practice, called creep feeding, has met with opposition within the equine industry because of fears that an excess intake of nutrients will result in excessive growth rates and an increase in developmental orthopedic disease. An alternative possibly however, is that the practice of maintaining growth rates prior to weaning, thereby reducing post-weaning compensatory growth (Lewis 1995), may actually reduce potential growth problems post-weaning.

Following weaning it is important to maintain an optimal growth rate in order to ensure that the young horse reaches it's genetic potential. In order to do this NRC (1989) suggests a diet that contains 30% forage and 70% concentrate. However, diets can be formulated using much higher levels of forage. These diets, particularly when the forage used is high quality alfalfa, which can contribute over 18% protein and .86% lysine (NRC 1989; Suleiman 1995), have nutrient to calorie ratios that are within NRC (1989) guidelines and supposedly meet the protein and lysine requirements. However, the availability of protein and lysine in the small intestine of high forage diets is a concern. Pre-cecal protein digestibility for forages has been reported to range from 2 to 58% (Hintz et al.1971; Gibbs et al. 1988). Because of this suspected lower pre-cecal availability even when high quality forages are fed it has been suggested that a readily available source of protein must be added to diets of horses which have a high protein requirements,(Glade 1983; Gibbs et al. 1988).

Hypothesis

The hypothesis examined in this thesis is that the protein and lysine in high quality alfalfa forage are not as available in the small intestine of the horse as are these components of concentrate, and therefore high forage diets formulated to meet NRC (1989) requirements will be deficient in these nutrients.

The specific objectives of this research were:

1) to demonstrate that creep feeding extensively managed foals will improve pre-weaning growth rate and to evaluate the effect of creep feeding on post-weaning weight loss,

2) to demonstrate that young growing horses can be fed high forage (>50% forage) diets, but that protein and lysine supplementation is required with such diets, and

3) to determine the pre-cecal availabilities of protein and lysine in forage and mixed forage:concentrate diets.

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CHAPTER 2

GROWTH AND CONDITION AT WEANING OF EXTENSIVELY MANAGED CREEP-FED FOALS

A. INTRODUCTION

When raising foals the goal is to produce a well developed, sound, blemish-free horse. Horse owners are continually looking for management practices conducive to raising young horses in a manner that meets the demands of today's horse industry. Creep feeding might be used to optimize growth in young horses and reduce stress at weaning[1].

Nutrients produced by the mare in early lactation adequately meet the requirements for the young, rapidly growing foal. However, by mid-lactation the nutrient secretion by a typical mare may not be sufficient to support optimal growth of the foal[2,3,4]. When creep feeding is practiced, the suckling foal's nutritional requirements for an optimal growth rate before weaning can be met and potential development problems associated with rapid compensatory growth following weaning can be reduced[5].

Foals are under a great deal of stress at weaning because their diet is changed, there is the loss of companionship with their dam and they may be moved to a new location. Stress at weaning can result in injury, weight loss and health problems[5]. These stresses can result in reduced competitiveness of the foals in the marketplace or in the show ring. Creep feeding can reduce stress on foals at weaning. Foals that are accustomed to dry feed prior to weaning exhibit less stress than those foals which do not receive concentrate before weaning[6]. McCall et al[7]. reported that creep feeding prior to weaning significantly reduced post-weaning stress in foals that were weaned using either a total or partial separation method of weaning.

This study was undertaken to evaluate the effects of creep feeding on the growth of extensively managed foals prior to weaning, and to determine the effect of creep feeding on weight loss and overall condition post-weaning.

B. MATERIALS AND METHODS

One hundred and four Quarter Horse, and Quarter Horse-type mares and their foals from a commercial breeding farm were used in a 53-day trial to study the effects of creep feeding on growth, condition and selling price of weanlings, and body weight and condition changes of their dams. The mare and foal pairs were assigned to 1 of 12 breeding herds containing 6 to 12 mares each at the direction of the farm manager. The herds were made up of mares of similar body weight that had been bred to Quarter Horse or American Paint Horse stallions the previous year. The breeding herds were then randomly assigned to one of three dietary treatments, with four herds per treatment, on July 22, 1997. The foals were 67 ± 16.3 (mean ± sd) days of age at the start of the trial.

The dietary treatments were no creep ration (NC), a pelleted, 13% protein creep ration (13%) or a pelleted, 17% protein creep ration (17%). The composition of the two creep feeds and concentrations of protein, digestible energy, calcium, and phosphorus in the two rations are found in Table 2-1. The creep rations were provided once daily at a rate of 0.45 kg/month of age per foal in a suitable creep feeder. Any feed not consumed was removed prior to the next feeding. The creep feeder consisted of a covered feed trough 3.6 m long x 0.75 m wide which was 0.75 m above the ground. The creep feeding area was enclosed by a 7.2 m x 7.2 m fence. Twenty vertical openings 0.36 m wide x 1.43 m high on each of two sides of the fence, allowed the foals access to the creep feeder. The creep feeder was located in each pasture in an area where the mares tended to congregate. These areas were generally well treed and close to the water source. In addition, salt/mineral stations were located in close proximity to the creep feeder to encourage the mares and foals to frequent this area. The mares and foals were maintained on pasture throughout the trial and mares did not receive any supplemental feed except salt and mineral. The 12 pastures were mixtures primarily of timothy, creeping red fescue, alsike clover and native grasses and ranged in size from 12 to 30 hectares. Stocking rates were 1.3 to 3 mare-foal pairs per ha.

Foals were weaned September 12 at 120 ± 16 (mean ± sd) days of age and sold at public auction on September 13. From weaning until sale time, all foals were housed indoors in groups of four to six foals. The creep-fed foals were fed the same concentrate they received during the summer plus mixed alfalfa-grass hay, while the NC foals received oats and alfalfa-grass hay. All groups had water free-choice.

To monitor the effects of treatment on growth performance, body weight and condition scores for mares and foals were determined at the start of the feeding period, on day 28 and at weaning (day 53), and at 24 hours post-weaning for the foals. Body weight was recorded using a livestock scale (Senstech 5000, manufactured by Senstech Ltd. Saskatoon, SK, Canada), body condition scores were obtained by two independent evaluators using the system of Henneke et al.[8] which ranges from 1-9. Feed efficiency was calculated using average feed intake for each of the creep-fed groups divided by the additional weight gained due to the creep feed. In addition, a general assessment score was taken on the foals 24 hours post-weaning. General assessment scores were obtained by two independent evaluators using a 1-5 system adapted from Griffin[9]; with a foal scoring 1 having a poor, rough hair coat and dull appearance while a foal scoring 5 having a soft smooth hair coat and a bright appearance.

Samples of the concentrate feeds were collected biweekly and composited to form a representative sample for the experiment. Samples were dried at 60°C for 96 hours, ground through a 1 mm screen and analysed for protein, calcium and phosphorus at a commercial feed testing laboratory (Norwest Laboratories, Edmonton, Alberta, Canada).

Pasture samples were collected August 20 (day 29 of the feeding period). Samples were taken by crossing each pasture on both diagonals and taking clippings each 30 meters. The clippings taken every 30 meters represented an area where the mares were grazing. Clippings were composited and four samples were taken from each pasture. Samples were stored on ice after collection then frozen at -20°C until analysed. The samples were dried at 60°C for 96 hours, ground through a 1 mm screen and analysed for dry matter (DM), crude protein, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus at the Soil and Crop

Diagnostic Laboratory, Alberta Agriculture, Food and Rural Development[10].

The growth data were examined by analysis of variance procedures for a split plot according to the methods of SAS[11] with treatment as the main effect and gender of the foal as the split. Treatment means were compared using the Student-Newman-Keuls test at P=0.05. Gender of the foal had no influence on any of the parameters measured, thus, gender differences are not shown in the data from the experiment. Regression analysis for factors that may have affected sale price (final body condition score, final body weight, age at weaning, registration status and general assessment score) was carried out using the stepwise regression procedure of SAS[11].

Feed intakes for the two creep-fed groups were analysed using a one-way analysis of variance procedure of SAS[11].

C. RESULTS

The pasture forage was of good quality (Table 2-2). Nutrient concentrations were similar between treatments except for calcium which was in the highest concentrations ($p<0.05$) in the NC treatment.

Initial body weights and condition scores for the broodmares were similar ($P>0.05$) for the three treatment groups (Table 2-3). The broodmares in all treatment groups gained weight over the experimental period, but there were no differences (P>0.05) in body weight, body condition score or gain at weaning.

Average age, average weight, and body condition scores of the foals at the start of the feeding period were not different (P>0.05)(Table 2-4).

Creep feed was offered at 0.45 kg/month of age per foal on a daily basis, such that the average amount of feed offered per foal ranged from 0.90 kg/day at the start to 1.35 kg/day at the end of the feeding period. Daily feed intakes (Table 2-4) were not different (P>0.05) for the two creep-fed groups (0.84 vs 0.56 kg/hd/day) and all groups had feed left over each day.

The average daily gains for 13% and 17% foals were 1.2 and 1.14 kg/day, respectively, both of which were greater (P<0.004) than gains for the non creep-fed foals (1.06 kg/day). The foals fed 13% or 17% rations had increases in body condition of 0.5 and 0.4 respectively, which were significantly higher (P<0.05) than the NC group.

The efficiencies of supplemental feed use for weight gain (feed:gain) for the 13% and 17% foals were 8.1 and 5.6, respectively, and were not different (P>0.05).

Post-weaning weight losses for the NC, 13% and 17% foals were 9.4, 6.8, and 8.1 kg, respectively (P=0.06). The general assessment scores were higher for the creep-fed foals, with scores of 2.5, 3.7, and 3.8 being obtained for the NC, 13% and the 17% foals respectively ($P<0.001$).

The stepwise regression for factors that could affect sale price resulted in general assessment score and final body condition score meeting the $P=0.05$ level of significance to enter the model (Table 2-5). The relationship of general assessment score and body condition score to sale price was significant($P<=0.0001$; $R^2= 0.51$).

D. DISCUSSION

While the NRC (1989)[2] does not give recommendations for specifications of creep feed, the concentrations of protein, calcium, and phosphorus for the 17% creep ration (Table 2-1) met or exceeded the concentrations suggested in NRC (1978)[12]. However, the 13% concentrate provided only 81, 75 and 90% of the protein, calcium and phosphorus, respectively, of the nutrient concentrations suggested by NRC (1978)[12]. The calculated digestible energy (DE) contents of both feeds were within suggested concentrations.

The analysis for nutrients in the pasture samples for all treatment groups were within ranges noted for mixed pasture forages in Alberta[13]. The difference in calcium content of the pastures was significant ($P<0.05$) and may be due to variation in the concentration of clover in the pastures. This difference would not be expected to affect differences between treatment groups for any parameter measured.

The nutritional quality of available forage allowed the mares to maintain body condition and gain an average of 0.21 kg/day while providing nutrients for the suckling foals. The weight gains of the mares in this trial were similar to those reported for Quarter Horse mares by Zimmerman[14] who suggested that mares should gain 0.15 - 0.35 kg/day body weight during the first 90 days of lactation to achieve maximum milk production. Actual creep feed intakes for the 13% and 17% groups were 0.84 and 0.56 kg/day, respectively, ($P>0.05$). It has been suggested that milk production of the mare will affect supplemental feed intake of the foals[14], therefore, the numerical differences in creep feed consumption, may have been due to differences in milk production by the mares or differences in feeding behaviour or quality of the two creep diets.

The creep diets were made up of oats and barley as the major grain components, while combinations of canola meal and soybean meal were used as the protein supplements. Soybean meal was used because it is the most common plant protein source used in horse rations[5], and canola meal because of its availability in Western Canada[15]. The use of either of these protein sources alone or in combination has not resulted in any negative effects on feed intake as reported by other researchers feeding young, growing horses[15,16]. However there are no reports in the literature on the acceptance of canola meal or soybean meal for foals of the age used in this study. Molasses was added to both diets to increase palatability, thus palatability of the feed should not have been a major factor in the intakes of feed.

In this trial, the proximity of the feed to where the mares were feeding could have played a role in daily creep feed intake. The mares and foals were maintained in large pastures resulting in infrequent visits to the creep feeder, in comparison to feeding situations where the foals eat with their dams while confined in a stall or are maintained in smaller paddocks which allow the foals ready access to the creep feed while still being close to their dams.

The intake of foals fed the 13% ration resulted in a slightly greater total intake of supplemental protein (109 vs 95 g/day) and energy (2.69 vs 1.80 Mcal DE/day) than the 17% fed foals. These differences in protein and energy intakes were not significant and not large enough to affect growth of the foals.

The growth rates of foals with an expected mature weight of 545 kg ranges from 1.20 kg/day at 2 - 3 months of age, decreasing to 1.05 kg/day by 4 months of age[5]. The non creep-fed foals had weight gains that are comparable to other reported values for non creep-fed foals. Breuer et al [17] reported an equation which can be used to predict rate of gain based on supplemental digestible energy intake: (Daily Gain kg/day) = 0.95 + 0.012 (supplemental DE MJ/d). Using this equation, the NC foals in this study would be expected to gain 0.95 kg/day, which is 0.11 kg/day lower than the actual gain. The additional DE required for this increase growth would need to be supplied by a higher level of milk production or forage intake. A daily intake of 1.0 kg of forage dry matter would supply the required DE to attain the growth rate of the NC foals and is well within the foal's digestive capacity. The growth rate of the creep-fed foals in this trial exceeded the estimation of Lewis[5] but are comparable to those reported by other researchers for Thoroughbred and Quarter Horse foals[17,18,19]. Using Breuer's equation the predicted daily gains for the 13% and 17% would be 1.08 and 1.03 kg/day respectively. These predicted gains are below what was actually achieved. As with the non-creep group a forage intake of approximately 1 kg of forage dry matter per day would provide the additional DE required to attain the weight gains recorded for the creep-fed foals.

The body weights for the foals in all treatment groups at weaning were comparable to those reported by other researchers for similar age foals[9,20,21]. The creep-fed foals had significantly greater gains in body condition during the feeding period while the non-creep groups maintained their initial body condition. The body condition scores of the 13% and 17% groups were similar to that reported for Thoroughbred foals of a similar age[21].

The efficiencies of use of creep feed for additional gain reported in this study (8.1 and 5.4)are comparable to those reported by others for four month old foals[5].

The stresses of weaning have been noted in the first 40 hours post-weaning. The effects of stress include a decrease in feed intake, a decrease in growth rate and an increase in susceptibility to disease[5]. It has been shown that creep feeding prior to weaning reduces stress[6,7]. It is probable that foals accustomed to eating dry feed prior to weaning maintained their feed intake through the early stages of the post-weaning period which tended (P=0.06) to reduce the amount of weight lost by the foals. The NC foals lost 9.4 kg while the 13% and 17% foals lost 6.8 and 8.1 kg, respectively, in the 24-hour period post-weaning. For the NC, 13% and 17% groups this amounted to 4.8%, 3.4% and 4.0% of weaned weight loss, respectively.

The foals in this trial that were fed prior to weaning had higher general assessment scores with shinier hair coats, and a general brighter appearance 24 hours post-weaning than the NC foals. The assessment scores for the creep-fed foals were slightly below those reported by other researchers[9].

It is generally accepted that horses offered for sale must be well presented to attract top value. The improvement in general assessment score and final body condition score (2.5, 5.5; 3.7,5.8; and 3.8,5.8 for the NC, 13% and 17% groups, respectively) resulted in an increase in sale price. Creep-fed foals with a general assessment score of 3.8 and a body condition score of 5.8 had a predicted sale price of $936.70 while the non creep-fed foals with a general assessment score of 2.5 and a final body condition score of 5.5 had a predicted sale price of $440.60, based upon the equation presented in Table 5. The increased sale price for the foals with higher general assessment scores and body condition scores results from being creep-fed prior to weaning. Although improved appearance can increase the value of horses at sales, buyers use other factors such as pedigree, color and correctness of conformation to make their final decisions in determining actual value.

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Table 2-1. Nutrient profile and analysis of the concentrate rations

	Protein in Creep Ration	
	13%	17%
Ingredients		
Oats %	43.0	48.0
Barley %	34.0	16.0
Corn %	5	---
Wheat %	---	5.5
Soybean Meal %	3.5	14.0
Canola Meal %	5.0	7.0
Molasses %	5.5	4.6
Vitamin Mineral Premix[A] %	4.0	4.9
Analyses (as fed)		
Protein %	13.0%	16.8
Digestible Energy Mcal/kg[B]	3.20	3.20
Calcium %	0.60	0.90
Phosphorus %	0.50	0.70

[A]Vitamin mineral premix used was Champion Feeds Vitamin Mineral premix[R]. Feeds were manufactured by Champion Feeds Ltd., Barrhead, Alberta, Canada and resembled 13% Groomer Horse Ration[R] and 17% Rite Start Foal Ration[R].
[B]Calculated from ingredient values.

Table 2-2. Average nutrient analysis of pasture forage (dry basis)

Nutrient	No Creep	Protein in Creep		SEM[A]	P
		13%	17%		
Dry Matter %	35.3	34.6	37.2	1.75	0.49
DE Mcal/kg[B]	2.30	2.32	2.31	0.13	0.49
Protein %	11.0	10.1	9.6	1.10	0.73
Acid Detergent Fiber %	33.8	32.6	33.3	0.8	0.57
Neutral Detergent Fiber %	56.9	59.6	60.2	1.22	0.24
Calcium %	0.64[a]	0.57[ab]	0.53[b]	4.03	0.05
Phosphorus %	0.16	0.14	0.13	0.02	0.62

[A]Pooled standard error based on four samples per treatment.
[B]DE Mcal/kg estimated from %ADF on dry matter basis; DE = (3.38-(0.2xADF%))x.85. Formula was derived from research at the Ruminant Forage Evaluation Unit, University of Alberta. The 0.85 correction factor is used to convert to DE for horses based on coefficients from Hintz (1969)[22].
[ab]Values within a row with unlike superscripts are significantly different ($P<0.05$).

Table 2-3. Least square means for mare body weight and body condition score (BCS)

		Protein in Creep			
	No Creep	13%	17%	SEM[A]	Probability
Number of Mares	38	30	36		
Initial body wt, kg	542	546	561	10.80	0.44
Body wt at weaning, kg	549	568	571	10.40	0.30
Daily gain, kg/day	0.06	0.38	0.18	0.10	0.14
Initial BCS	5.9	5.6	5.5	0.19	0.44
Final BCS	6.0	6.0	5.8	0.14	0.47

[A]Pooled standard error.

Table 2-4. Least square means for foal body weight, average daily gains (ADG), body condition score, daily feed intake, post weaning weight loss, general assessment score and sale price for a 53 day feeding period

		Protein in Creep			
	No Creep	13%	17%	SEM[A]	P
Number of animals	38	30	36		
Age, days					
Initial	63	69	67	4.5	0.55
Final	116	122	120	4.5	0.55
Body weight, kg					
Initial	137	137	144	4.3	0.41
Final	192	199	204	3.5	0.12
Daily gain	1.06^b	1.20^a	1.14^a	0.02	0.004
Creep feed intake kg/hd/d	0.0	0.84	0.56	0.16	0.25
Feed efficiency[B]	--	8.1	5.6	1.89	0.38
Body condition score					
Initial	5.5	5.3	5.4	0.13	0.60
Final	5.5	5.8	5.8	0.10	0.12
Change	0.0^b	0.5^a	0.4^a	0.13	0.05
Post-weaning					
Weight loss, kg	9.4	6.8	8.1	0.65	0.06
General assessment score	2.5^b	3.7^a	3.8^a	0.13	0.0001
Sale price, $ Cdn	384.00^b	945.00^a	937.00^a	74.3	0.0006

[A] Pooled standard error.

[B] Calculated as kg creep feed per kg of additional gain above non creep-fed foals

[ab] Values within a row with unlike subscripts are significantly different($P<0.05$).

Table 2-5. Regression analysis for factors related to sale price[A]

	Sale Price	SE	P
Intercept	-1697.4	371.6	<.01
Regression Coefficient			
General Assessment Score (b_1)	326.0	39.2	<.01
Body Condition Score (b_2)	240.5	67.9	<.01
R^2 Values			
General Assessment Score	0.454		
Body Condition Score	0.061		
Total	0.515		

[A]No other variable met the 0.05 level of significance for entry into the model.

CHAPTER 3

THE EFFECT OF PROTEIN SUPPLEMENTATION OF ALFALFA CUBE DIETS ON THE GROWTH OF WEANLING HORSES

A. Introduction

The nutrient requirements for the young growing horse depend on age, weight and expected growth rate. The NRC (1989) suggests that to meet the nutrient requirements of weanling horses, diets should contain 30% forage with the remaining portion made up of concentrate[1]. However, diets for the young growing horse can be formulated to meet nutrient requirements using high quality forage at levels of up to 70% of the diet. These diets, particularly when alfalfa is used as the forage, have nutrient to calorie ratios that are within the NRC (1989) guidelines and supposedly meet the requirements for crude protein (CP) and lysine. However, availability of protein and lysine in high forage diets has been the focus of debate. Pre-cecal protein digestibility has been reported to range from 2% to 58%[2,3]. This has led to the suggestion that a source of available protein must be added to forage-based diets of horses which have high protein requirements even with the use of high quality forage[3,4].

The experiment described in this report was designed to determine if dietary protein and lysine concentrations need to be increased when young growing horses are fed diets containing 60% alfalfa forage.

B. Materials and Methods

Sixty weanling fillies of stock horse type, approximately 5 months of age, and with an average weight of 220 kg (± SD 17.6 kg) were used in a 141-day growth trial which commenced November 15, 1995. Foals were maintained in the research facility on a diet of long stem hay and rolled grain for 21 days prior to the start of the trial. The foals were stratified by weight and randomly allocated within weight groups to one of 12 pens with five animals per pen and four pens per dietary treatment. Body weight measurements were taken on 2 consecutive days at the beginning and end of the trial, and on days 28, 70, and 98. Body condition scores[5] were taken at the end of the trial.

Dietary treatments contained 60% alfalfa cubes and 40% concentrate with varying dietary CP concentrations. The control treatment (15% CP) was formulated to just meet the nutrient requirements for a 220 kg weanling with an expected mature size of 525 kg and gaining 0.85 kg/day. The other diets (17% CP and 19% CP) were formulated to provide additional protein and lysine (Table 3-1). The dietary treatments were formulated to be isocaloric and other nutrients met or exceeded NRC (1989) requirements.

Horses were fed once daily. Amounts of feed offered were adjusted to allow ad libitum feed intake to a maximum intake of 3% of body weight on an as-fed basis. Maximum feed allowances were adjusted after each weigh period. Feed provided was recorded daily on a per pen basis, and feed refusals were recorded weekly. Water was available ad libitum from automatic waters. Fortified trace mineral salt with copper, manganese, and zinc concentrations of 2500 mg/kg, 3500 mg/kg, 7500 mg/kg, respectively was provided free choice.

The horses were housed in 6 m x 17 m dry lot pens with a 20% porosity wind fence on the west side of the pens. Wood chips were provided for bedding as required.

Samples of the three concentrate rations, and the alfalfa cubes were taken daily and composited for each period. Feeds were analysed for dry matter (DM), CP, lysine, acid detergent fibre (ADF), calcium and phosphorus using the standard procedures of the Alberta Agriculture, Food and Rural Development, Soil and Crop Diagnostic Laboratory[6].

Daily gain, dry matter intake, and feed efficiency data were analysed as a split plot analysis of variance, with time as the split plot using the General Linear Model (GLM) procedures of SAS[7]. Treatment means were compared using the Student-Newman-Keuls test at P=0.05.

C. Results and Discussion

The analysis of the alfalfa cubes and the three concentrate mixtures are shown in Table 3-1. The analysis of the alfalfa cubes were similar to reasonable quality early-bloom alfalfa hay (NRC 1989). Use of alfalfa cubes rather than alfalfa hay would not affect nutrient availability in this experiment since maturity has a greater effect on nutrient availability than processing[8].

Least square means for average daily gain (ADG), dry matter intake (DMI), feed efficiency (feed:gain) and body condition score are reported in Table 3-2. There were no differences (P>0.05) for any of the parameters measured nor were there any significant interactions between these parameters and time on feed.

During the first period the horses given the 15% CP, 17% CP and 19% CP diets consumed 803, 877 and 979 g protein daily which was equivalent to 100, 110 and 122%, respectively, of their protein requirements according to NRC (1989). Corresponding intakes of lysine were 100, 112 and 124% respectively. The lower than expected protein concentration in the 19% CP diet resulted in intakes lower than expected. The daily intakes of protein and lysine increased throughout the trial as daily feed intakes increased and met or exceeded the treatment goals (data for periods 2-4 not reported). The foals throughout the trial consumed on average 1050, 1126 and 1238 g protein daily for the 15% CP, 17% CP and 19% CP diets, respectively. Corresponding values for lysine were 43, 48, and 55. These protein and lysine intakes all met or exceeded the NRC (1989) requirements for growth of 0.85 kg/day.

The addition of protein and lysine above NRC (1989) levels in this trial therefore did not give a growth response. Other researchers have reported that the addition of protein above requirements does not produce an increase in daily gain with weanlings and yearlings fed diets that provide recommended levels of digestible energy[9,10]. In research with weanlings fed diets that were 65% concentrate, 848 g of protein daily were adequate for 0.76 kg/day gain in the study of Ott and Asquith (1983)[11]. Cymbaluk (1990) reported that lower intakes of protein (780 g/d) and lysine (24.8 g/d) resulted in a reduction in the growth rate of 6-8 month old draft horse foals when digestible energy intakes were constant[12]. The results of this trial and others would suggest that the addition of a supplemental protein source is not needed to meet the protein requirements of the young horse fed forage-based diets (> 50% forage), which contradicts the suggestion of Gibbs et al.(1982)[3]. Further work is therefore needed to determine the intestinal digestibility of protein in high forage diets. However, lack of response to protein and lysine in our study could have been because cold weather increased energy requirements without increasing protein requirements. The lower growth rates achieved in this trial (.70-.72 kg/day) could be due to a lower level of digestible energy in the diets than predicted or may have been due to climatic conditions (cold temperatures). Thompson et al. (1988) reported that foals had lower growth rates than predicted as a result of colder temperatures and suggested that protein and energy must be increased proportionally to increase growth rate[13].

Daily dry matter intake (DMI) ranged from 5.2 to 7.8 kg per horse per day for the duration of the feeding period (Table 3-3). These values correspond to intakes of 2.5% body weight and are comparable to reported values by other researchers using diets with a minimum of 50% forage[10,14,15]. As the horses grew, there was a significant increase (P<0.001) in DMI from period one through period four (Table

3-3) which is consistent with results of Cymbaluk et al. (1989)[16].

The growth rate for all dietary treatments exceeded moderate growth rate indicated by NRC (1989) for a weanling with an expected mature weight of 525 kg. This rate of growth is comparable to those reported by other researchers for weanlings fed diets containing more than 60% concentrate[11,13,17,18]. Cymbaluk and Christison (1989), using diets containing 70% forage, reported gains with weanlings ranging from 0.66 kg/day to 0.81 kg/day[14]. Finnish workers, using diets contained 60% grass hay, reported gains of 0.47 kg/day to 0.52 kg/day[10]. Rate of gain was lower in period four than in the first three periods (Table 3-3). Similarly, Cymbaluk (1989) reported decreased growth rates as stock horse weanlings matured[19].

The feed conversions of 9.8 to 10.0 kg D.M. per kg gain are similar to values found in previous trials with high forage diets[20]. Ott and Asquith (1983) reported slightly better feed conversions (8.3 to 9.3) for weanlings fed diets containing 60% concentrate[11]. Feed efficiencies changed significantly over the feeding period, with the best feed conversion being recorded in period one (6.97) and the feed conversions in periods two and three not differing (9.56, 9.65, respectively). Reduced gain in period four resulted in the poorest feed:gain ratio (13.3:1). As the young horse matures, composition of weight gain changes. There is a corresponding reduction in protein requirement due to a decrease in protein content of gain and an increase in fat content of gain. This change reflects a decrease in efficiency of digestible energy use.

Body condition scores were used to indicate relative body composition. Body condition scores found in this trial were similar to values reported by Topliff et al. (1988) for weaning Quarter Horses, and Pagan et al. (1996) for Thoroughbreds of similar age[17,21]. It would appear that the daily gain was of similar composition for the fillies in this trial regardless of the level of protein supplementation.

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Table 3-1. Nutrient Analysis[1] and Ingredient Composition of the Concentrate Mixes and Alfalfa Cubes

	Concentrate Mixtures				
	15% CP	17% CP	19% CP	Alfalfa Cubes	SEM[4]
Ingredients					
Oats %	38.6	38.2	38.1		
Barley %	53.2	43.8	32.3		
Soybean Meal %	---	10.5	22.9		
Canola Oil %	5.8	5.3	4.8		
Mineral/Vit	2.38	2.20	1.90		
Diet Composition					
DM %	89.35	90.0	89.64	85.63	0.39
DE[2] Mcal/kg	3.77	3.76	3.75	2.35	0.01
Protein %	12.0	15.26	19.04	18	0.43
Lysine %	0.38	0.60	0.85	0.81	0.03
ADF[3] %	10.49	10.77	10.06	29.69	0.70
Ca %	0.74	0.60	0.55	1.84	0.04
Phos %	0.72	0.72	0.70	0.24	0.016

[1]All values on a dry matter basis.
[2]DE Mcal/kg calculated using NRC 1989 values.
[3]ADF - Acid Detergent Fiber.
[4]SEM - standard error of the mean based on four samples of feed.
[5]Mineral/Vitamin Premix made up of 31% salt, 66% dicalcium phosphate and 3% vitamin ADE premix with vitamin A at 10,000,000 IU/kg.

Table 3-2. Least Square Means by Treatment for Average Daily Gain, Dry Matter Intake, Feed Efficiency and Body Condition Score

	Treatment				
	15% CP	17% CP	19% CP	SEM[1]	P
Number of Animals	20	20	20	---	---
Body Weight kg					
Initial wt	222	219	220	1.32	0.26
Final wt	321	315	320	4.50	0.56
ADG[2] kg/day	0.72	0.70	0.71	0.032	0.85
DMI[3] kg/day	6.73	6.66	6.72	0.069	0.74
Feed:Gain Ratio	9.82	10.0	9.84	0.387	0.89
BCS[4]	5.43	5.20	5.45	0.097	0.19

[1]Standard error of mean based on 20 weanling fillies per mean.
[2]Average Daily Gain
[3]Dry Matter Intake
[4]Body Condition Score BCS - based on the 1-9 system of Henneke et al. (1983)

Table 3-3. Least Square Means by Period for Daily Gain, Dry Matter Intake and Feed Efficiency

	Period[1]					
	1	**2**	**3**	**4**	**SEM [2]**	**P**
ADG [3], kg day	0.76[a]	0.71[a]	0.76[a]	0.61[b]	0.023	0.0001
DMI [4], kg day	5.18[a]	6.66[b]	7.28[c]	7.78[d]	0.056	0.0001
Feed:Gain Ratio	6.97[a]	9.56[b]	9.65[b]	13.37[c]	0.49	0.0001

[1] **Period 1** Nov 15/95 - Dec 12/95, **Period 2** Dec 13/95 - Jan 23/96
Period 3 Jan 24/96 - Feb 20/96, **Period 4** Feb 21/96 - Apr 3/96

[2]Standard error of mean
[3]Average Daily Gain
[4]Dry Matter Intake

[abcd] Least square means with unlike superscripts within a row are significantly different at P<0.0001.

CHAPTER 4

CANNULATION OF THE DISTAL ILEUM IN PONIES

A. Introduction

Cannulation of the gastrointestinal tract allows sampling of digesta at specific points of the tract for determination of the role of the different segments of the tract in nutrient digestion and absorption. The cannulation techniques and design of cannulae for use in ruminant digestion studies have been recently reviewed by Harmon and Richards (1996). In the equine, cannulation of the digestive tract has been successful in the cecum and large intestine (Baker et al. 1969; Lowe et al. 1970; Wilkins and Lowe 1993). Cannulation of the small intestine has been limited, however different techniques for cannulation have been reported by Peloso et al. (1994), Gerhards et al. (1991), and Roberts and Hill (1974). In the reports on cannulation techniques there is little information provided on how long cannulated animals survive and what day-to-day care is required. Roberts and Hill (1974), using a cannula machined from perspex, reported that ponies were used for 82 d. Peloso et al. (1994) report ponies being healthy at 1.5 mon post-surgery but give no indication as to the length of time animals with a silastic cannula were used.

Improvements in cannulation techniques and cannula design will help to reduce post surgery complication, increase the life-span of the cannulated animal, and ensure the collection of reliable data. The purpose of this report is to describe the techniques for cannulation of the distal ileum of ponies using a plastisol cannula with an internal diameter of 2.5 cm, and to describe the routine maintenance required for cannulated animals.

B. Materials and Methods

Polyvinyl chloride (Plastisol - CA1098 Clear, F.H. & Sons MFG Ltd., Rexdale, ON) was selected as the material to construct the cannula. Plastisol is relatively inexpensive, flexible, and easily used for manufacturing. The design for the cannula was adapted from one used in swine (Dr. W. Sauer, personal communication). The simple "T" cannula was constructed on a stainless steel mold. The mold was 16.5 cm long with a 14.5 cm "T" stem and had an outside diameter of 2.5 cm. The cannulae were formed by heating the stainless steel mold for 45 min at 250°C in an electric furnace (Blue M - Model No. CHH-16C, Blue M Electric Co., Blue Island, Illinois), completely immersing the mold in plastisol for 5 min, reheating at 250° for 4 min, then re-immersing for 1 min in the plastisol. The mold encased in plastisol was then returned to the electric furnace and heated at 210°C for up to 25 min or until the color of the plastisol changed from white to dark yellow. Following heating, the cannula was allowed to cool, cut from the mold and shaped into a simple T-cannula (Figure 1). All edges were rounded and sanded smooth. The resulting cannula had a 16.5 cm base, a 12 cm stem, and an internal diameter of 2.5 cm. A PVC plug (2.5 cm non metallic schedule 40 female adapter, Ramon Home Products) with a 1.25 cm plastic screw cap (purchased at a local hardware store, Revy Home and Garden, Edmonton, AB) was inserted in the stem portion of the cannula to act as a stopper. A 10 cm diameter plexiglass washer with a 3.9 cm center hole, and a size 20 hose clamp were used to secure the cannula following surgical installation.

Ponies used in this procedure were mature, Welsh type geldings with an average weight of 252 kg ± 31 (mean ± SD). The ponies ranged in age from 4 to 10 yr. All ponies were acclimated to the Laird McElroy Metabolic Research Unit, University of Alberta, and dewormed with an anthelmintic (Eqvalan-ivermectin, Merck Agvet, Kirkland, PQ.) prior to surgery.

Care of the animals and procedures used were in accordance with the Canadian Council on Animal Care guidelines and approved by the Faculty Animal Policy and Welfare Committee, Faculty of Agriculture Forestry and Home Economics, University of Alberta.

The surgical procedures were adapted from Peloso et al. (1994). Daily feed intakes were gradually reduced over 4 d prior to surgery. This was done to reduce potential digestive upsets due to rapid diet changes. Twenty-four h before surgery, the ponies were transported to Edmonton Equine Veterinary Services where the surgeries took place. Once at the veterinary clinic, all feed was withheld for 12 h before surgery.

Pre-operative preparation included placement of a 14 gauge, 5.0 cm catheter in the right jugular vein, clipping the right flank, and administration of procaine penicillin (40,000 IU/kg^{-1} body wt intramuscularly (IM); Pfizer Inc., Montreal, PQ.), flunixin meglumine (0.5 mg/kg^{-1} body wt intravenously (IV); Vetrapharm London ON.).

Anesthesia was induced with ketamine HCL (1 ml per 45 kg body wt IV; Ayerst Montreal PQ.) followed by sedation with xylazine HCL (0.5 ml per 45 kg body wt IV; Bayer Etobicoke ON.)and glycerol guaiacolate (10% solution wt vol^{-1} at 1 ml per 2 kg body wt IV; BDH Toronto ON) and maintained with halothane vaporized in oxygen.

The ponies were placed in left lateral recumbancy and the right abdomen prepared for surgery. A 15 cm vertical incision through the skin and cutaneous trunci muscle was made midway between tuber coxae and the last rib. Using a grid technique, the external and internal abdominal oblique and transverse abdominal muscles were divided along the direction of their fibers. The retroperitoneal fat was bluntly separated and the peritoneum incised with scissors. The small intestine was exteriorized and the antimesenteric band of the ileum located. The exteriorized portion of the ileum was packed in moist towels. Contents of the exteriorized portion of the ileum were stripped into the cecum. With the intestine slightly stretched in a longitudinal direction, a 5 cm incision was made into the intestine on the antimesenteric surface of the ileum. The base of the cannula was folded at the stem and both ends were simultaneously inserted into the incision. Starting at the oral end of the incision, the ileal stoma was sutured until the stem of the cannula fit loosely against the aboral end of the incision. The ileum was anchored to the stem of the cannula using 20 interrupted sutures around the circumference of the cannula stem. The sutures included a full thickness bite of the ileum and a partial bite of the cannula at the stem base junction. The screw top was placed into the stem of the cannula and the cannulated ileum was replaced into the abdomen. To create a stoma for exteriorization of the stem of the cannula, a 2.5 cm circular section of skin and cutaneous trunci muscle was removed from the abdomen cranial to the fold in the flank 6 cm caudal to 16^{th} rib. Scissor blades were forced through the body wall and peritoneum into the body cavity in the center of the circular defect. The cannula was exteriorized by traction on the umbilical tape which was secured to the stem of the cannula. The ileum was palpated to ensure it was properly aligned. The cannula and ileum were pulled in close contact with the body wall and secured in position with the plexiglass washer and hose clamp. The internal and external abdominal oblique muscles and the subcutaneous tissue of the paralumbar incision were closed separately using a simple continuous pattern.

The ponies were recovered with supervision to prevent problems during recovery. Within 4 h of recovery the ponies were looking to eat, and within 12 h were eating and had passed fecal material. Trimethoprin Sulfa (sulfamethoxazole 800 mg and trimethoprin 160 mg per tablet; Apotex Inc, Toronto ON.) was administered orally for 10 d following surgery.

C. Results and Discussion

Postoperatively, the ponies were kept at the veterinary clinic for 5 d then returned to the Laird McElroy Metabolic Research Unit. During the recovery period the ponies were maintained on a high forage diet with pelleted grain introduced 10 d post-surgery. The external sutures were removed 14 d post-surgery. Ponies were not allowed to exercise freely because of concerns about dislodging the cannula, instead, all ponies were hand-walked for 20 min for the first 2 wks post-surgery then subsequently exercised for 45 min daily by either hand-walking or on a mechanical walker. The stoma was well formed by 28 d following surgery.

At one month post-surgery, all ponies were healthy and the cannulas working. With one pony which had exhibited a tendency to chew on the cannula, a neck cradle was used to prevent him from dislodging the cannula. Two ponies of the five which were cannulated for the first experiment had their cannulas dislodge for unknown reasons (one pony had displacement occur at 63 d post-surgery while the second had displacement occur on d 87 post-surgery). Attempts to replace the cannulae in both instances were unsuccessful and resulted in the need to euthanize the ponies. Post-mortem results indicated a well formed stoma with no signs of peritonitis or intestinal damage. The post-mortem results did not provide any answers as to why the cannulae dislodged. It is possible that these two ponies pulled their cannulas out due to discomfort. In subsequent cannulations of four animals the weight of the cannula was reduced by shortening the exteriorized portion of the stem. In addition, the washer and clamp were removed 28 d post-surgery to reduce discomfort due to pressure on the ileum and stoma. No problems were noted once these procedural modifications were made.

There was some leakage around the cannula (three of nine ponies) as reported by others with cannulated animals (Peloso et al. 1994; Roberts and Hill 1974). This leakage was not excessive and did not cause any noted health problems. Because of this leakage, daily cleaning of the cannula area was required.

Gerhards et al. (1991) reported the presence of granulation tissue around the cannula and potential build-up of intraluminal granulation tissue. Granulation tissue formed around the cannula on two of the nine ponies but did not affect the functioning of the cannula. Intraluminal granulation tissue was not seen in the two ponies that were euthanized.

Colic has been reported by other researchers as a complication with ileal cannulated ponies (Peloso et al. 1994). Mild colic was noted in one pony during the recovery period, this was treated with 10 cc IM Dipyrone (50% Vetoquinol Canada Inc., Joliette, PQ.) and 5 cc IM Flunixin meglumine (Vetrapharm London ON.) as required. Other ponies (three of nine) in this report experienced colic 5 to 6 wks post-surgery. The colic was intermittent, mild in nature, and did not appear to be related to the surgical procedure.

The placement of the cannula on the right side in the paralumbar region was similar to the placement previously reported (Peloso et al. 1994). Location of the cannula allowed easy access for sample collection and routine maintenance of the cannula area. All ponies accepted handling of the cannula for routine cleaning and sample collection.

The cannulation of the distal ileum was easily performed. The plastisol cannula (2.5 cm internal diameter) allowed for easy collection of ileal contents on a wide variety of diets with varying forage concentrate ratios. One of the original cannulated ponies has remained useful at over 500 d post-surgery. Ponies from the second group that were cannulated remained functional for over 90 d post-surgery.

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Legend

A Screw Top
B Plug
C Hose Clamp #20
D Plexiglass Washer - 13 cm diameter
E Base of Cannula
F Stem of Cannula

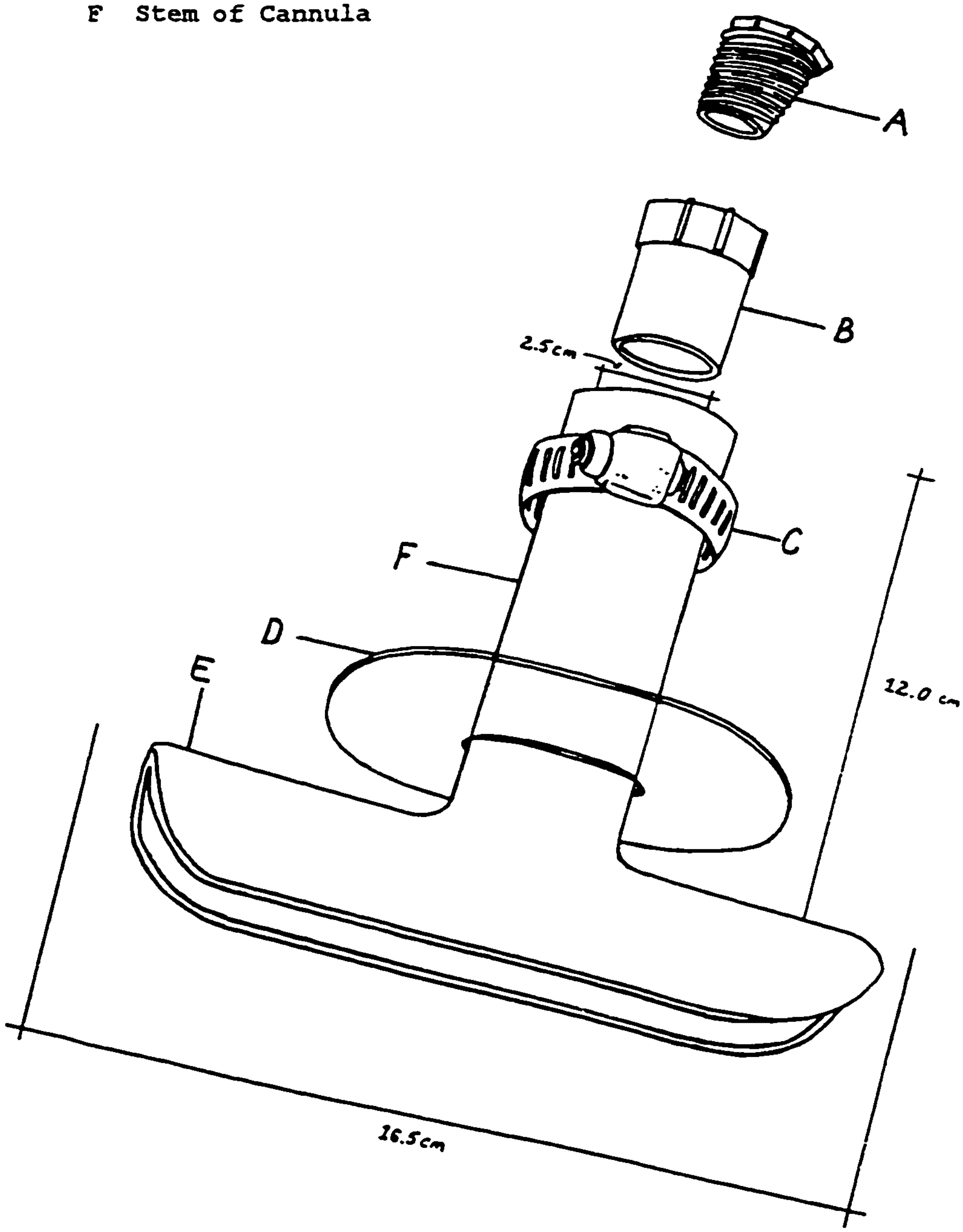

FIGURE 4-1 ILEAL CANNULA

CHAPTER 5

A COMPARISON OF CHROMIUM-MORDANTED HAY FIBER, CO-EDTA, AND TOTAL FECAL COLLECTION AS METHODS FOR DETERMINING APPARENT DIGESTIVE COEFFICIENTS IN HORSES

A. Introduction

The use of external markers as reference compounds to monitor aspects of nutrient digestion is well documented. Chromic oxide (Cr_2O_3) has been the most frequently used external marker in equine nutrition research. Apparent digestion coefficients determined using Cr_2O_3 compared favorably with coefficients determined from total collection in studies of Parkins et al. (1982) and Todd et al. (1995). However, Sauer et al. (1979) reported that using Cr_2O_3 as a marker significantly underestimated digestion coefficients with one of four diets when compared to the total collection method. The lower coefficient was due to a reduced recovery of the marker with the feeding of a low fiber diet. Only one experiment was found in which chromium-mordanted hay fiber has been used as a solid phase digesta marker. Results were favorable in this case (Cuddeford and Hughes 1990). There are no experiments with horses in which a liquid phase marker such as Co-EDTA has been examined as a potential marker for digestibility studies.

The use of a two marker system has been suggested in ruminants to mark the liquid and solid phase of intestinal digesta (Faichney 1980). A two marker system, with Co-EDTA to mark the liquid phase and Cr_2O_3 to mark the solid phase, has been used for the determination of digesta flow rate in the horse (Nyberg et al. 1995). Despite their popularity with ruminant researchers there are no reports in which Co-EDTA and Cr-mordanted hay fiber have been used as dual markers in equine research.

We have used a two marker system to study ileal digestibilities in companion studies (Chapters 6 and 7) which necessitated the measurement of marker recoveries which are reported in this paper.

B. Materials and Methods

In this report the use of chromium-mordanted hay fiber as a particulate phase marker and Co-EDTA as a liquid phase marker were compared for determining apparent total tract digestion coefficients in diets fed to mature ponies.

In exp. 1, four mature pony geldings with an average weight of 267 kg (± 34 kg SD) were used. These ponies were cannulated in the distal ileum according to the procedures of Coleman et al. (1998). Dietary treatments were 1) 100% alfalfa cubes which contained 19% crude protein (CP), 2) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 15% CP (15% CP), 3) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 17% CP (17% CP) and 4) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 19% CP (19% CP). The alfalfa cube and mixed diets were fed at 1.6% and 1.3% of body weight (DM basis), respectively, and met the digestible energy requirements for maintenance (Chapters 4,6 and7).

In exp. 2 (unpublished observations), four mature pony geldings with an average weight of 234 kg (± 12.3 kg SD) were used. As with exp. 1, these ponies were cannulated in the distal ileum. Dietary treatments were 1) 100% alfalfa cubes, 2) 75% alfalfa cubes and 25% concentrate, 3) 50% alfalfa cubes and 50 concentrate, and 4) 25% alfalfa cubes and 75% concentrate. The diets were fed at 1.6% body weight on a DM basis and provided equal protein intakes across all treatments .

The following procedures were used in both experiments. Each period in the Latin square consisted of a 10 d adaption and 4 d total collection. During the collection period, ponies were maintained in collection stalls that allowed for separate collection of urine and feces. Care of the animals and procedures used were in accordance with the guidelines of the Faculty Animal Policy and Welfare Committee, Faculty of Agriculture Forestry and Home Economics, University of Alberta.

Diets were fed three times daily with markers added to the concentrate portion of each meal (with the 100% hay cube diets, the markers were added to 50 g of concentrate mix). External markers used were cobalt complex of ethylenediamine tetraacetic acid (**Co-EDTA**) and chromium-mordanted hay fiber (**Cr-hay fiber**). The markers were made following the procedures of Udén et al. (1980). The liquid phase marker Co-EDTA (14.3% Co), was fed at 9 g d^{-1} and the particulate phase marker Cr-hay fiber (5.2% Cr),was fed at 30 g d^{-1} in exp. 1 and 24 g d^{-1} in exp. 2.

Feed samples were taken daily and composited to provide one sample per pony per period. Total fecal material was weighed daily over the 4 d period and a 10% subsample taken for the composite sample to provide one sample per pony per period. All feed and fecal samples were dried at 60°C for 96 hours and ground in a Wiley mill to pass a 1 mm screen. Cobalt and chromium were determined using procedures of Reese et al. (1994).

Data were analysed using two-way analysis of variance using GLM procedures of SAS (SAS Institute 1990). Comparison of least square means was done using pdiff procedures of SAS. In addition a paired "t" test was done comparing the dry matter digestion coefficients determined by each marker method with total collection determinations. Since probabilities with the paired "t" test were similar to those obtained using the analysis of variance procedures, only the analysis of variance results are reported. The recoveries of Cr-hay fiber in exp. 2 were further analyzed using the REG procedures of SAS (SAS Institute 1990).

C. Results and Discussion

The overall recovery rates of Co-EDTA and Cr-hay fiber in the feces were 94.7% and 95.5% (exp. 1)and 87.1% and 95.7% (exp. 2) respectively (Table 5-1). The recovery rates for both markers were corrected for the marker removed with the ileal samples. In both exp. 1 and exp. 2 there was no interactions or apparent trends for the diet to affect recoveries of Co-EDTA reported in Table 5-1. However a quadratic regression analysis for data in exp. 2 showed a significant ($P<0.01$) diet effect for the recovery of the Cr-hay fiber; as the level of forage in the diet decreased to 50% and 25%, recoveries decreased to 93.2 and 92.3% respectively (Fig. 5-1).

The recoveries of Cr-hay fiber in exp.1 (95.5%) and recoveries of Cr-hay fiber in diets containing 75 and 100% forage in exp. 2 (98.6 and 98.7%) are similar to recoveries with particulate markers reported in other studies. Haenlein et al. (1966) reported Cr_2O_3 recovery rates of 98.4% with all-forage diets when the marker was given orally in a gelatin capsule prior to each meal. Parkins et al. (1982) also used the gelatin capsule method for marker administration. The capsule was given daily and subsequent recoveries were 95%. In studies where the markers were fed as a top dress, recoveries reported were 100% for Cr_2O_3 (Todd et al. 1995) and 96.5% for Cr-hay fiber (Cuddeford and Hughes 1992). Thus on the basis of our results and the literature cited above the percentage recovery of Cr markers in feces averaged 97.0 ± 1.7 (mean ±SD; n=8) when diets containing more than 60% forage are fed. The apparent losses of marker may be caused by either incomplete fecal collections or loss of marker from the feed or the digestive tract. The losses from the digestive tract would include a small loss when the cannula barrel was voided prior to ileal samples being taken and any leakage of digesta from the cannula site. In either case the errors resulting from incomplete recoveries when diets contain more than 50% forage are small, relatively unimportant, and need not be corrected for. However high recoveries for Cr with forage diets are not always obtained; Knapka et al. (1967) using Cr_2O_3 reported a recovery of 81.5% with burros when the marker was hand mixed into individual meals.

When diets containing 50 and 25% forage were fed in exp. 2 Cr-hay fiber recoveries were 93.2 and 92.2% respectively. There is other evidence that marker recovery may be lower with low fiber diets. When the Cr_2O_3 marker was incorporated in a pelleted diet, as in the trial of Sauer et al. (1979) recovery was 95.4% with three of four diets, whereas a lower recovery (76.9%) was noted when ponies were fed a low fiber diet. The authors suggested that the reduction in marker recovery was caused by irregular feed intakes on the low fiber diets. In contrast with such problems reported by Sauer et al. (1979), there were no apparent irregularities in feed intake in our study, thus the decrease in recovery with low fiber diets may have been due to an increase in marker absorption caused by factors such as intestinal pH or a greatly lengthened retention time of the marked particles in the cecum. Howell and Cupps (1950) reported that retention time of digesta in the cecum was reduced when alfalfa forage was included in the diet. Recoveries of marker may therefore have been improved if a marker administration period of 5 d or more had preceded the collection period.

The recovery of Co-EDTA marker was 94.7% in exp. 1, however, the overall recovery was only 87.1% in exp. 2. There are no comparable reports in the literature in which Co-EDTA has been used as a digestibility marker in horses.

It has been reported in ruminants that the absorption of 1 - 5% of the dosed Co-EDTA occurs and must be corrected for (Owens and Hanson 1992). While some absorption may have occurred in our studies, it would not account for the low recovery of the Co-EDTA in exp. 2. The soluble nature of the Co-EDTA allowed the marker to stick on ponies muzzles, and on the feeders during meal consumption resulting in a reduced intake of the marker. These observations were not noted during exp. 1 where ponies tended to consume the concentrate portion of their meals faster and subsequent recovery rates of both markers were similar. Incomplete recovery of marker can result in an overestimation of fecal output and a subsequent underestimation of digestion (Haenlein et al. 1966).

Digestion coefficients for dry matter are found in Table 5-1. As might be expected from data on marker recoveries, there were no significant differences ($P>0.05$) between digestion coefficients determined by total fecal collection, Cr-hay fiber, or Co-EDTA methods in exp. 1. In exp. 2, there were no significant differences ($P>0.05$) between overall dry matter digestion coefficients determined using the total collection (68.5%) or Cr-hay fiber (67.7%) methods, however the dry matter coefficient determined using Co-EDTA (62.6%) was significantly ($P<0.05$) lower.

In conclusion, the use of chromium-mordanted hay fiber as a solid phase marker provided reasonable estimates of apparent total tract dry matter digestibility of high forage diets(>60% forage). However reduced recoveries of marker in diets with low levels of forage can result in underestimation of the digestion coefficients when compared to the total fecal collection method. Co-EDTA did not give reproducible results.

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Table 5-1. Least square means for marker recovery and percentage digestibility of dry matter determined by total fecal collection (TC), chromium-mordanted hay fiber (Cr-hay fiber), or Co-EDTA (Co) digesta markers.

	Marker Recovery (%)				Dry Matter Digestibility (%)				
	Cr-hay fiber	Co-EDTA	SEM[z]	P[y]	TC	Cr-hay fiber	Co-EDTA	SEM[z]	P[y]
Experiment 1									
Overall	95.5	94.7	1.68	0.63	65.2	63.7	63.8	1.10	0.55
Alfalfa Cubes	95.0	95.9	3.11	0.78	61.0	58.1	62.0	2.03	0.79
Mixed diet, 15% CP	91.8	89.3	3.59	0.78	68.1	65.1	64.3	2.34	0.79
Mixed diet, 17% CP	97.3	98.8	3.11	0.78	67.5	68.4	67.3	2.03	0.79
Mixed diet, 19% CP	97.4	92.9	3.59	0.78	64.3	63.0	61.8	2.34	0.79
Experiment 2									
Overall	95.7[a]	87.1[b]	1.38	<0.01	68.5[a]	67.7[a]	62.6[b]	0.79	<0.01
100% Alfalfa Cubes	98.6	91.8	2.65	0.08	61.9[a]	65.9[a]	56.9[b]	1.53	0.03
75% Alfalfa Cubes	98.7[a]	84.3[b]	2.65	<0.01	66.2[a]	67.7[a]	60.2[b]	1.53	0.03
50% Alfalfa Cubes	93.2[a]	83.4[b]	2.65	0.02	71.8[a]	69.6[a]	64.5[b]	1.53	0.03
25% Alfalfa Cubes	92.2	89.1	3.07	0.48	74.2[a]	67.5[b]	68.8[b]	1.77	0.03

[z] SEM - in exp 1, treatment NRC and NRC 130 n = 3, in exp 2, 25% alfalfa cubes n = 3, all other treatments n = 4.
[y] P = probability.
[ab] Values within the same row and comparison not followed by the same superscript are significantly different (p>0.05).

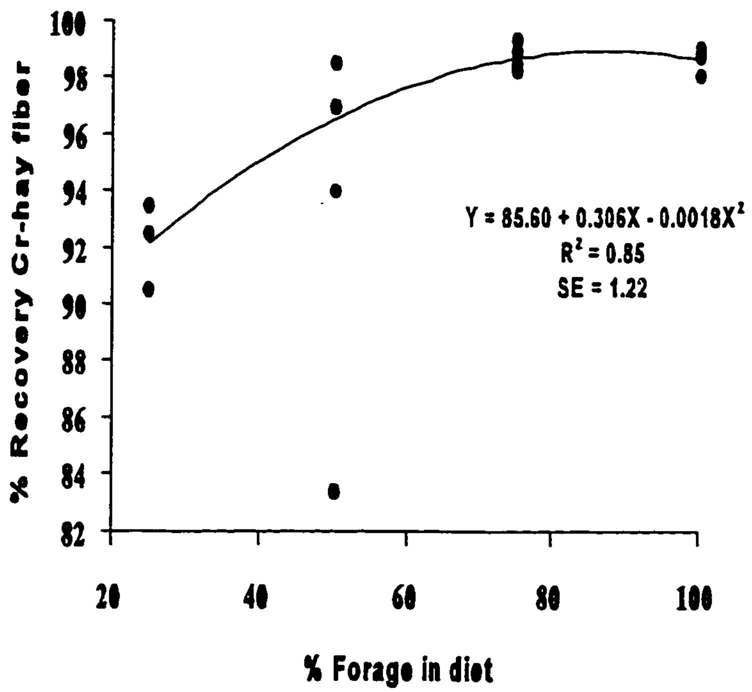

Fig. 5-1 Relationship of the recovery of Cr-hay fiber to percentage forage in experiment 2 (one data point for the 50% forage diet was not included in regression relationship)

CHAPTER 6

A COMPARISON OF SINGLE AND DUAL PHASE MARKERS FOR DETERMINING Pre-cecal APPARENT DRY MATTER DIGESTION COEFFICIENTS IN HORSES

A. Introduction

When using a single marker the assumption is made that samples which are taken are representative of the digesta (Titgemeyer 1997). However it has been reported that when chromic oxide (Cr_2O_3) was used as an external marker for horses, maximum marker concentration was recorded 30-60 min prior to maximum dry matter (DM) concentration (Haley 1981). Moreover, if samples of ileal digesta obtained are not representative of material flowing past the ileum at the time of sampling there may be over- or under-estimation of flow and digestibility.

The use of a dual phase marker system has been recommended as a means of correcting problems with unrepresentative samples. The dual phase system uses one marker which associates with the liquid phase of the digesta, while a second marker associates with the particulate phase of the digesta. Composition of true digesta is then determined mathematically based on the marker concentrations in the two phases of digesta (Faichney 1980). The use of cobalt EDTA (**Co-EDTA**), as the liquid phase marker and chromium mordanted hay fiber (**Cr-hay fiber**) have been suggested as reasonable markers to use in a multiple marker system (Udén et al. 1980). Faichney (1980) suggests that markers in a dual marker system do not need to associate exclusively with one phase. However, it has been suggested that Cr_2O_3 may not be an ideal marker because it does not specifically associate with any portion of the digesta (Udén et al. 1980; Titgemeyer 1997).

In this report, the use of **Cr-hay fiber** as a particulate phase marker and **Co-EDTA** as a liquid phase marker were compared as single phase markers and contrasted with a dual marker procedure for the determination of apparent pre-cecal dry matter **(DM)** digestion coefficients in diets fed to mature ponies.

B. Materials and Methods

Two experiments were conducted with ponies in which estimations were made of the amount of DM flowing past the ileum. Care of the animals and procedures used were in accordance with the guidelines of the Faculty Animal Policy and Welfare Committee, Faculty of Agriculture Forestry and Home Economics, University of Alberta.

In exp. 1, four mature pony geldings with an average weight of 257 kg (± 33 kg SD) were used. These ponies were cannulated in the distal ileum according to the procedures of Coleman et al. (1998). Dietary treatments were 1) 100% alfalfa cubes which contained 19% crude protein (CP), 2) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 15% CP (15% CP), 3) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 17% CP (17% CP) and 4) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 19% CP (19% CP). The cubed hay diet was fed at 1.6% of BW while the mixed diets were fed at 1.3 % BW (DM basis) and met the digestible energy requirements for maintenance (Chapter 4,5 and 7).

In exp. 2, four mature pony geldings with an average weight of 234 kg (± 12.3 kg SD) were used. As with exp. 1, these ponies were cannulated in the distal ileum. Dietary treatments were 1) 100% alfalfa cubes, 2) 75% alfalfa cubes and 25% concentrate, 3) 50% alfalfa cubes and 50 concentrate, and 4) 25% alfalfa cubes and 75% concentrate. The diets were fed at 1.6% body weight on a DM basis and provided equal protein intakes across all treatments (unpublished results).

The following procedures were used in both experiments. Each period in the Latin square consisted of a 10 d adaption and 4 d total collection. During each collection period, ponies were maintained in collection stalls that allowed for separate collection of feces and urine.

Diets were fed three times daily with markers added to the concentrate portion of each meal (the markers were added to 50 g of concentrate mix with the 100% hay cube diets). External markers used were Co-EDTA and Cr-hay fiber which were made following the procedures of Udén et al. (1980). The liquid phase marker Co-EDTA (14.3% Co), was fed at 9 g d^{-1}, the particulate phase marker Cr-hay fiber (5.2% Cr),was fed at 30 g d^{-1} in exp. 1 and 24 g d^{-1} in exp. 2.

Ileal samples were taken at 4 h intervals over a 4 d period. The samples were taken in random order to represent each hour of a 24 hr period starting 30 min after the 0800 h feeding on d-1. During each collection the cannula barrel was voided, and 175 mL snap-lid containers were used to collect digesta over a 4 min timed interval. Very occasionally an excessive amount of digesta was obtained and the digesta was collected for less than 4 min. Samples were immediately frozen at -20°C for further analysis. Following each period, ileal samples were thawed, mixed thoroughly and a 20% by weight alliquot from each individual collection was taken to provide one composite sample per pony per period. A second 20% by weight alliquot from each individual collection was taken to provide a second composite sample. This sample was centrifuged at 2900 x g for 30 min to extract the liquid phase, thus providing a single liquid phase sample for each pony for each period.

Feed samples were taken daily and composited to provide one sample per pony per period. All feed samples were dried at 60°C for 96 hours and ground in a Wiley mill to pass a 1 mm screen. Ileal samples were dried in an industrial freeze dryer (Virtis Model 40264-A, Virtis Co., New York) at -60°C for 14 days, and ground in a Wiley mill to pass a 1 mm screen. Samples were analysed for DM at the Soil and Crop Diagnostic Laboratory (Anonymous 1996). Cobalt and chromium were determined using procedures of Reese et al. (1994).

Ileal dry matter digestibility coefficients for the Cr-hay fiber and the Co-EDTA markers were determined using the relationship: Flow = daily dose/marker concentration(Owens and Hanson 1992). Marker intakes were corrected for fecal recoveries as determined in Chapter 5.

After recalculating all marker concentrations as a fraction of the original marker dose per g DM, ileal DM digestion coefficients were calculated using the dual marker technique of Faichney (1975;1980) as follows:

If x = a quantity of digesta (D)

y = a quantity of fluid (F) which, when added to or removed from x, reconstitutes true digesta (TD)

S_D, S_F, S_{TD} = concentrations of the solute marker in digesta, fluid, and true digesta, respectively.

P_D, P_F, P_{TD} = concentrations of the particle marker in digesta, fluid, and true digesta, respectively.

then $(x.S_D)+(y.S_F)= (x.P_D)+ (y.P_F)$

so that $Y/X = (P_D-S_D)/(S_F-P_F)= R$

where R is the reconstitution factor (i.e. the number of units of fluid that must be added to or removed from one unit of digesta to obtain true digesta).

then $(S_D+R.S_{FD})/(1+R)= S_{TD} = (P_D+R.P_F)/(1+R) = P_{TD}$

and flow of $TD = 1/S_{TD} = 1/P_{TD}$

Data were analysed by analysis of variance using GLM procedures of SAS (SAS Institute 1990). Comparison of least square means was done using PDIFF procedures of SAS.

C. Results and Discussion

Overall recovery rates of Co-EDTA and Cr-hay in feces were 94.7% and 95.5% (exp.1) and 87.1% and 95.7% (exp.2) respectively. Therefore marker dosage was corrected using fecal recovery as discussed in the previous Chapter. This practice is consistent with the recommendation of Faichney (1980) who suggested that marker dose be corrected to account for losses due to marker absorption. Similarly Titgemeyer (1997) indicated that fecal recovery is an appropriate estimator of marker intake when fecal recovery is incomplete.

Ileal digestion coefficients for DM are found in Table 6-1. There were no significant differences ($P>0.05$) between overall coefficients determined by the dual phase marker, Cr-hay fiber, or Co-EDTA methods in exp. 1 or exp. 2. Similarly, in both exp. 1 and exp. 2 there were no interactions between diet and the marker method used to determine digestibility. However, in exp. 2, while not significant, there was a trend ($P=0.06$) for the Cr-hay fiber to give different results than the Co-EDTA marker. Numerically, the dual phase system was between the particulate phase marker and the liquid phase marker. A similar non-significant effect occurred in exp.1.

The similarity in results using the different marker procedures could be due in part to the consistency of the samples. The reconstitution factor (R) that is calculated with the dual marker technique allows for the addition or subtraction of the fluid phase from the digesta to give a sample that represents true digesta (Faichney 1980). The R values for exp. 1 and exp. 2 were 0.039 ± 0.066 and 0.023 ± 0.028 (mean ± SD) respectively. These values are below the 0.07 value reported by Faichney (1980) with abomasal sampling in sheep. The low R values would suggest that the samples taken from the ileum are representative of the digesta flowing past the cannula. During each collection, the cannula barrel was voided and this material was discarded. The digesta that was collected generally came as a gush of material rather than a slow irregular flow thus it would be expected to be representative of true digesta.

The results of the two experiments reported here indicate that DM digestibilities estimated using single markers were similar to those estimated with the dual marker technique. Titgemeyer (1997) reported that use of a single marker system would produce satisfactory results when representative samples are collected which appears to have been the case in these experiments. In addition, however, similar results with particulate and liquid markers can only be expected if a sufficient number of samples are taken daily since timing of the flow of the particulate and liquid phase from the stomach is different in horses. Argenzio et al. (1974) reported, using the liquid phase marker polyethelene glycol, that the fluid marker had reached the cecum of ponies within 30 min of dosing, while 75% of the particulate marker polyethelene tubing (OD 2mm) was still in the stomach. Similarly Haley (1981) reported that when Cr_2O_3 was used as the marker, maximum marker concentration was noted at the ileum of ponies 30-60 min before maximum DM concentration. Because of the differential flow of the liquid and particulate phases, it is important to take samples in such manner that the entire period is equally represented. Owens and Hanson (1992) suggest that by sampling throughout the day, variation will be reduced and the sample would be representative of the digesta flowing past the cannula. From the above discussion it can be concluded that sampling intervals should be such that the entire 24 hr period is sampled and the sampling frequency should represent each time period post-feeding equally. It would appear from our results that a sampling interval of 1 h was adequate for the ponies and the feeding levels used in our experiments.

Since there did not appear to be a need to use the dual marker method in these experiments, we would recommend that Cr-hay fiber be used as a single phase ileal flow marker for horses. A soluble marker such as Co-EDTA is not recommended since fecal recoveries of Co-EDTA were consistantly less than Cr-hay fiber in exp. 2 and fecal recoveries were more variable (Chapter 5). The soluble nature of the Co-EDTA resulted in marker sticking to the muzzles of ponies and sides of feeders during exp. 2. Similar problems were not seen in exp. 1, where ponies tended to consume their concentration portion of the diet faster and recoveries of both markers were similar.

In conclusion, estimates of ileal digestibility were similar whether Co-EDTA, Cr-hay fiber or a dual marker procedure was used to estimate ileal flow. It is therefore suggested that Cr-hay fiber be used as a marker and that a sufficient number of samples be taken at equally spaced intervals throughout the day for accurate estimations of ileal flow in the horse.

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Table **6-1**. Least square means for percentage digestibility of dry matter at the ileum determined by chromium mordanted hay fiber (Cr-hay fiber), Co-EDTA, or the dual marker technique

	Number of Observations	Dual Marker	Cr-hay fiber	Co-EDTA	SEM[z]	P[y]
Experiment 1						
Alfalfa cubes	4	24.9	26.6	23.4	1.22	0.51
Mixed diet 15% CP	3	35.8	38.1	36.3	1.41	0.51
Mixed diet 17% CP	4	33.9	36.3	34.0	1.22	0.51
Mixed diet 19% CP	3	35.4	33.7	35.7	1.41	0.51
Overall	14	32.5	33.7	32.4	0.66	0.30
Experiment 2						
100% Alfalfa cubes	4	23.3	24.3	22.8	0.97	0.97
75% Alfalfa cubes	4	30.2	32.4	29.7	0.97	0.97
50% Alfalfa cubes	4	36.8	37.4	36.6	0.97	0.97
25% Alfalfa cubes	3	38.4	39.8	38.1	1.12	0.97
Overall	15	32.2	33.5	31.8	0.51	0.06

[z] SEM pooled standard error of mean.
[y] Probability

CHAPTER 7

EFFECT OF DIETARY FORAGE AND PROTEIN CONCENTRATION ON TOTAL TRACT, Pre-cecal AND POST-ILEAL DIGESTIBILITY OF FORAGE-BASED DIETS FED TO MATURE PONIES

A. Introduction

The NRC (1989) suggests that for young growing horses with high protein requirements, forage should make up only 30% of the diet with the remaining portion being concentrate. However, diets for young growing horses can be formulated with high quality forage at levels of up to 70% of the diet. These diets, particularly when alfalfa is used, supposedly meet protein and lysine as well as energy requirements. High forage diets (>50% forage) have successfully been used for young growing horses (Coleman et al. 1997), however, the availability of protein and lysine in the small intestine with high forage diets is of concern. Because of the possible low pre-cecal availability of protein and amino acids in forages, it has been suggested that even when high quality forages are used, a source of available protein must be added to diets of horses which have high protein requirements (Glade 1983; Gibbs et al. 1988).

In the small intestine, enzymatic digestion of protein results in amino acids being available for absorption, while the nitrogen in protein digested in the large intestine is absorbed mainly as ammonia (Potter et al. 1992). Apparent pre-cecal protein digestibility in all-forage diets has been reported to range from 2% to 58% (Hintz et al. 1971; Gibbs et al. 1988). The differences in pre-cecal protein digestibility in these studies may be due to the experimental procedures used. Hintz et al.(1971) used a slaughter technique to determine digestion coefficients in the different segments of the digestive tract, while Gibbs et al.(1988) used ponies cannulated in the distal ileum.

The objective of this study was to determine the apparent pre-cecal, post-ileal and total tract digestion of protein and lysine in forage-based diets fed to mature ponies fitted with a simple "T" cannula in the distal ileum.

B. Materials and Methods

Animals, Diets and Total Tract Digestibilities

A 4x4 Latin Square design experiment was conducted to determine the apparent digestibility of DM, CP, lysine, ADF and NDF in different segments of the digestive tract in horses fed a cubed alfalfa diet and three forage:concentrate diets containing graded levels of protein and lysine. The experiment used five mature pony geldings with an average weight of 257 kg (± 33 kg SD). The ponies were cannulated in the distal ileum according to the procedures of Coleman et al. (1998). Dietary treatments were 1) 100% alfalfa cubes which contained 19% CP, 2) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 15% CP (15% CP), 3) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 17% CP (17% CP) and 4) a mixed diet of 60% alfalfa cubes and 40% concentrate containing 19% CP (19% CP). The alfalfa cube diet was fed at 1.6% and the mixed diets were fed at 1.3% BW

(DM basis) and met the DE requirements for maintenance. Rations were fed three times daily at 8 h intervals. Nutrient analysis for the alfalfa cubes, the concentrate mixtures and the concentrate formulations are found in Table 7-1.

Each period of the Latin Square consisted of a 10 d adaption followed by a 4 d collection period. During the collection period, the ponies were maintained in collection stalls that allowed for separate collection of urine and feces. The ponies were acclimated to the collection stalls for 24 hrs prior to the start of each collection period. Samples of the concentrate feeds and the alfalfa cubes were taken daily and composited to form a representative sample of each feed for each period. Total fecal material was weighed daily over the 4 d period and a 10% by weight sub-sample was taken for the composite sample to provide one sample per pony per period. All feed and fecal samples were dried at 60° for 96 hr and ground in a Wiley mill to pass a 1 mm screen. Care of the animals and procedures used were in accordance with the guidelines of the Faculty Animal Policy and Welfare Committee, Faculty of Agriculture, Forestry and Home Economics, University of Alberta.

Measurements of ileal flow

Commencing on d 7 of the adaption period the digesta flow markers were added to the concentrate portion of each meal (with 100% hay cube diets, the markers were added to 50 g of concentrate mix). External markers used were cobalt complex of ethylenediamine tetraacetic acid (**Co-EDTA**) and chromium-mordanted hay fiber (**Cr-hay fiber**). The liquid phase marker Co-EDTA (14.3% Co), was fed at 9 g d^{-1} and the particulate phase marker Cr-hay fiber (5.2% Cr), was fed at 30 g d^{-1}. The markers were made following the procedures of Udén et al. (1980).

Ileal samples were taken at 4 h intervals over the 4 d collection period. The samples were taken in random order to represent each hour of a 24 hr period starting 30 min after the 0800 h feeding on d-1. During each collection the cannula barrel was voided, and 175 mL snap-lid containers were used to collect digesta over a 4 min timed interval. Very occasionally an excessive amount of digesta was obtained and the digesta was collected for less than 4 min. Samples were immediately frozen at -20°C for further analysis. Following each period, ileal samples were thawed, mixed thoroughly and a 20% by weight alliquot from each individual collection was taken to provide one composite sample per pony per period. A second 20% by weight alliquot from each individual collection was taken to provide a second composite sample. This sample was centrifuged at 3000 x g for 30 min to extract the liquid phase, thus providing a single liquid phase sample for each pony for each period. Ileal samples were dried in an industrial freeze dryer (Virtis SRC model 40264A, Virtis Co., New York, NY) at -60°C for 14 days. Following drying, all samples were ground to pass a 1 mm screen in a Wiley mill.

Chemical analysis

Samples were analysed for DM, CP, lysine, ADF and NDF at the Soil and Crop Diagnostic Laboratory (Anonymous 1996). Samples were analyzed for gross energy using a Leco AC300 Automatic Calorimeter (Leco Corporation, St. Joseph, MI).

Chromium and cobalt were determined using the procedures of Reese et al.(1994).

Calculations

Total tract digestion coefficients for DM, CP, lysine, ADF and NDF were calculated based on total collection of feces. Although single and dual marker procedures gave similar results (Chapter 6), pre-cecal and post-ileal digestion coefficients for DM, CP, lysine, ADF, and NDF were determined using the dual marker procedures of Faichney (1975;1980) as follows:

If x = a quantity of digesta (D)

y = a quantity of fluid (F) which, when added to or removed from x, reconstitutes true digesta (TD)

S_D, S_F, S_{TD} = concentrations of the solute marker in digesta, fluid, and true digesta, respectively.

P_D, P_F, P_{TD} = concentrations of the particle marker in digesta, fluid, and true digesta, respectively.

then $(x.S_D)+(y.S_F) = (x.P_D)+(y.P_F)$

so that $Y/X = (P_D-S_D)/(S_F-P_F) = R$

where R is the reconstitution factor (i.e. the number of units of fluid that must be added to or removed from one unit of digesta to obtain true digesta).

then $(S_D+R.S_{FO})/(1+R) = S_{TD} = (P_D+R.P_F)/(1+R) = P_{TD}$

and flow of $TD = 1/S_{TD} = 1/P_{TD}$

Statistical Analysis

The original design of this experiment was a 4x4 Latin square. However, due to complications with the cannlae that resulted in ponies being removed from the trial and the addition of an extra pony and period, the resulting data were analysed as a completely randomized design, with treatments, periods and animals as sources of variation (SAS 1990). Orthogonal contrasts were not used with the forage:concentrate diets due to very similar protein and lysine intakes for the 17% CP and the 19% CP diets. Preliminary analysis indicated that period had no effect on the parameters measured so period was removed from the model. However there were major differences among ponies for pre-cecal protein and pre-cecal and post-ileal lysine digestibilities. Therefore for these parameters only, treatments and animals were included in the model as sources of variation. Where significant differences were noted due to treatment effects, least square means were tested using the PDIFF procedure of (SAS 1990). True digestibility of protein and lysine in the different segments of the digestive tract were determined for the 15, 17 and the 19% CP diets using the REG procedures of SAS (1990).

C. Results and Discussion

Animals

The ponies used in this experiment were cannulated in the distal ileum following the procedure reported in Coleman et al. (1998). Due to problems with ponies dislodging cannulas, two ponies were removed from the trial, one pony at the end of period one and the second at the end of period 2. An additional pony was added in period 2. It is of interest that all ponies completed four experimental periods in a later experiment (unpublished results) using a similar cannulation procedure.

Feed and Intake

Analysis of the alfalfa cubes suggests that the forage was comparable to an early bloom alfalfa hay (NRC 1989). Although the 15% CP and the 17% CP concentrate mixtures were within expected range for protein and lysine levels, the protein and lysine analysis for the concentrate mixture in the 19% CP diet (i.e. 18.1% and .78%, respectively Table 7-2) were below the expected values of 20.0% and .95%.

Feed and nutrient intakes (g/kg BW) are reported in Table 7-2. The DM intakes were higher with the alfalfa cubes but similar across the three forage:concentrate treatments. Calculated DE intake for the alfalfa cube diet was 9.3 Mcal DE/d, while the forage:concentrate diets provided a calculated DE intake of 9.5 Mcal/d. Crude protein intakes were highest (P=.02) with the alfalfa cube diet, while the intakes of 2.40 and 2.46 g/kg BW for the 17% CP and 19% CP diets, respectively, were not different (P>.05) due to a lower than desired concentration of protein in the 19% CP concentrate mix. Lysine intakes were highest with the forage diet and increased (P=.02) across the concentrate treatments. The ADF and NDF intakes were higher with the alfalfa cube diet but were similar across the mixed diets due to the consistent 60:40 forage concentrate ratios.

Total Tract Digestibilities

Total tract DM digestibilities were lower (P=.001) with the alfalfa cube diet (60.2%), than for the mixed diets (66.7%; Table 7-3). The DM digestibilities did not differ between the 17% CP and the 19% CP diets but there was a significant difference (P<.05) between the 15% CP and the 19% CP diets. The DM digestibility of the alfalfa diet was similar to the 61.4% and the 58.3% reported by Todd et al. (1995 a,b), while other reported values have been slightly lower. Hintz et al. (1971) noted a total tract DM digestibility of 55% with pelleted alfalfa, while Gibbs (1982) reported total tract DM of 42 and 52% for low protein(15%) and high protein (18%) alfalfa hays, respectively.

Our results with the mixed diets (66.7% DM digestibility) are similar to other reported digestibilities. Schurg and Holtan (1979) and Householder (1978) reported DM digestibilities of 62% for diets that had a 50:50 forage to concentrate ratio. Hintz et al. (1971) reported a DM digestibility of 69.6% with pelleted diets with a 60:40 forage to concentrate ratio. However, Thompson et al. (1984), using alfalfa oat diets in a 60:40 ratio, reported a lower DM digestibility of 56.7% with mature horses.

Total tract ADF digestibilites were 41.5, 38.0, 41.4 and 45.7% for the alfalfa cube, 15% CP, 17% CP and the 19% CP diets, respectively. Comparative values for NDF digestibilities were 43.5, 43.8, 43.8 and 39.6%. There were no differences (P>.05) in digestibility of either type of fiber due to dietary treatment. These results are similar to the limited reports on total tract digestibility of ADF and NDF in equine diets. Schurg (1981) reported apparent ADF digestibilities of 43 to 45% for alfalfa hay, while Todd et al. (1995) noted an NDF digestibility of 37.4%. Hintz et al.

(1971), with pelleted alfalfa, reported an NDF digestibility of 41.2%. In mixed forage: concentrate diets, Ott (1981) noted an ADF digestibility of 41.4% for horse fed a diet of 50% forage, while Schurg and Holtan (1979) reported an apparent digestibility of ADF of 44% for horses fed a 50:50 forage:concentrate diet. Thompson et al. (1984) using diets with a 60:40 forage:concentrate ratio reported a lower ADF digestibility of 32%. The apparent digestibility of NDF reported by Hintz et al. (1971) for 60:40 forage:concentrate diet was 54.8%, while Webb et al.(1985) with a 50:50 forage:concentrate diet reported digestibilities of 34% and 40% for Quarter Horses and Miniature Horses, respectively.

The total tract protein digestibility with the alfalfa cube diet was 71.9% which is similar to literature values of 65 to 78% (Haenlien et al. 1966; Hintz et al. 1971; Schurg 1981; Todd et al. 1995). Gibbs et al. (1988) reported N digestibilities of 73.8% for an 18% CP hay while the 15% CP hay nitrogen digestibility was 66.1%. The average total tract protein digestibility of the mixed diets in this study was 75.4% (Table 7-3). As the level of protein increased in the mixed diets there was a trend to increased apparent protein digestibility. The increase in apparent total tract protein digestibility may have been due to the increase in total protein intake and a diluting effect of metabolic fecal nitrogen as reported by Slade and Robinson (1970). Protein digestibility in our experiment was greater than the 66.7% reported by Householder (1978) with 50:50 forage:concentrate diets containing 9.8% CP, and much greater than the 56.3 reported by Ott (1981) with mixed diets containing 7% CP. The differences are due to the low protein content of these diets; in this trial the forage was a high quality alfalfa, while the two aforementioned reports bermuda grass was the forage used. Fonnesbeck et al. (1967) reported that nutrient digestibilities were higher with alfalfa forage than with grass forages. Hintz et al. (1971) using diets with 60:40 or 20:80 forage:concentrate ratios and 17.5 and 16.9 % CP reported protein digestibilities of 75.5 and 79.8%, respectively.

Total tract digestibility of lysine in the alfalfa diet (71.6%) was 90% of that observed in the 19% CP mixed diet (78.7%). There was a trend (P<.1) to increasing digestibility as the level of lysine increased in the concentrate. As with the total tract protein digestibilities, it appears that apparent total tract lysine digestibility may increase as the lysine intake increases, which is to be expected if there is a relatively constant excretion of metabolic fecal lysine. While there have been numerous research reports on the effect of lysine supplementation in the diet for the young growing horse, there are no reports on lysine digestibility in the horses. Fan et al. (1995) reported lysine digestibility of 80.8% for early weaned pigs fed a diet using soybean meal as the protein source.

Pre-cecal Digestibilties

The pre-cecal DM digestibilities were 23.4, 35.7, 33.9, and 35.4 (P=.0001) for the alfalfa cube, 15% CP, 17% CP and the 19% CP diets, respectively. Protein concentration did not affect (P>.05) pre-cecal DM digestibility in the mixed diets. Hintz et al. (1971) reported an apparent pre-cecal DM digestibility of 18.1% for pelleted alfalfa while

Gibbs (1982) noted pre-cecal DM digestibilities of 1.8% to 8.2% for chopped alfalfa hay. Our results with mixed diets are intermediate between the two other sets of results available in the literature. With mixed diets, Hintz et al. (1971) reported pre-cecal DM digestibilities of 24 and 36% for diets with 60:40 and 20:80 forage to concentrate ratios, respectively. Researchers in Texas using ileal cannulated ponies have reported pre-cecal DM digestibilities of 46.0% (Haley 1981), 53.6% (Householder 1978), 51% (Arnold 1982) and 61.4% (Gibbs 1982) with diets with forage:concentrate ratios ranging from 50:50 to 25:75. Differences between these two research groups could be due to procedures used. Hintz et al. (1971) used a slaughter technique in determining partial tract digestibilities while the other researchers used ileal cannulated ponies or horses. In either procedure, ensuring that a representative sample is taken is critical. Hintz et al. (1971) remarked that sampling from a different site within in the specific section of the digestive tract would affect the results in their procedure. In sampling from a cannula, there is the concern that the sample taken is truly represents the digesta flowing past the cannulation site. In the horse, it has been reported that the liquid phase of digesta flows rapidly through the small intestine such that liquid phase markers reach the cecum 30 min post-dosing (Argenzio et al. 1974). However, the movement of the particulate phase is much slower. Hintz (1990) noted that, while some of the particulate phase may reach the cecum in 30 min, feed particles can remain in the stomach for up to 7 hrs. In the research of Arnold (1982) and Gibbs (1982), ileal digesta was sampled frequently for the first 120 to 240 min post dosing then at a reduced frequency until the next meal, while in the research of Haley (1981) and Householder (1978) samples were taken at 30 and 15 min intervals for 5 and 6 h post feeding, respectively. In our trial, ileal samples were taken over a constant time interval to represent each hr of a 24 hr period and then combined on the basis of 20% of individual sample weight to form a composite sample. Therefore, our samples should be representative of the daily digesta flow and average daily marker concentration in digesta. Our procedure is consistent with the recommendation of Owens and Hanson (1992) who suggested that samples be taken throughout the 24 h feeding period to ensure the sampling protocol yields a representative sample.

Sampling protocol could have a large influence on estimates of apparent digestibility. For example, Haley (1981) reported that the maximum concentration of chromic oxide reached the terminal ileum 30 to 60 min prior to the maximum concentration of DM. In this situation a protocol with more frequent sampling immediatly after dosing would result in an underestimation of flow past the ileum and hence an overestimation of DM digestibility. In contrast, concentration of sampling times at the time of peak DM flow could result in overestimation of ileal flow. Differences in DM digestibility estimates obtained due to timing of sampling could also be amplified if the sample taken at the ileum is not representative of the digesta flowing past the ileum at that particular time. Fiachney (1980) has suggested that the use of a dual marker technique to overcome this problem. We have compared a dual marker system with a single marker for determining ileal flow and obtained no difference between the two techniques (Chapter 6). This suggests that the proportion of liquid and particulates in our ileal samples were representative of what was flowing past the ileum at the time of sampling. Since our cannulation technique was similar to that of Peloso et al. (1994), with the exception that a cannula with a slightly larger internal diameter was used, this would suggest that the use of a single vs a dual marker system was not the reason for the differences between our results and those reported by other researchers using cannulated animals.

The pre-cecal digestibilities for ADF ranged from 6.2% to 11.8% and did not differ between diets. Pre-cecal digestibility of ADF has not previously been reported in the horse. The values reported here are low as may be expected because of the lack of the enzymes required to digest the components of fiber in the small intestine of the horse. The NDF pre-cecal digestibilities were 16.2, 17.5, 11.2, and 13.4 for the alfalfa cube, 15% CP, 17% CP and 19% CP diets, respectively. The NDF digestibility reported here is similar to that reported by Hintz et al. (1971). As with the work of Hintz et al. (1971), it appears that diet composition has little effect on the pre-cecal digestion of ADF or NDF.

Pre-cecal protein digestion in the forage:concentrate mixtures averaged 55% and, with the exception of the alfalfa cube diet, increased (P=.001) with dietary protein concentration. Pre-cecal protein digestibility with the alfalfa cube diet(47.8%) was less (P=.0001) than with the mixed diets. The pre-cecal protein digestibility for the alfalfa in this study is slightly higher than the pre-cecal protein digestibility of 41.1% for a pelleted alfalfa as reported in the study of Hintz et al. (1971) when a slaughter procedure was used. However, it is much higher than the values of 1.3 and 21.0% for 15% and 18% protein alfalfa hays, respectively as reported by Gibbs et al. (1988) using ileal cannulated ponies. The reported values of other researchers for mixed diets are variable. Hintz et al. (1971) reported pre-cecal protein digestibilities of 39.9 and 44.4% for 60:40 and 20:80 forage:concentrate ratio diets, respectively, which are lower than our results. The only other results which are available are from ileal cannulated horses used in the Texas research. Brown (1987) reported a lower pre-cecal protein digestion of 24% with 30:70 forage:concentrate diets fed at 1.5% BW. These diets were fed in two, three, or four meals per d but frequency of feeding had no effect on digestibility. Other Texas researchers have reported pre-cecal nitrogen digestibilities in mixed diets from 50.8% (Haley 1981), 53.6% (Householder 1978) for 50% forage diets and 46.6% (Gibbs 1982) for 25% forage diets. Farley et al. (1995) reported apparent pre-cecal N digestibilities of 36.7 to 59.6% with diets containing graded levels of soybean meal and 4.9 to 16.5% crude protein.

The pre-cecal protein digestibility in the concentrate portion of our diet can be calculated on the basis of the digestibility of the protein in the alfalfa cube diet and the proportion of alfalfa in the 19% CP diet since the protein concentrations in both diets were similar. Using this methodology, the estimated pre-cecal digestibility of the protein in the concentrate was 79%. The alfalfa cube diet therefore had a pre-cecal protein digestibility that was 61% of the concentrate.

It has been suggested that the lower pre-cecal availability of protein in forage necessitates the use of a highly digestible source of protein in diets for horses with high protein requirements (Glade 1983; Gibbs et al. 1988). Coleman et al. (1997) reported that there was no growth response in 220 kg weanling fillies gaining .70 kg/d with increased protein concentration when mixed diets from this study were fed. Using pre-cecal protein digestibilities of 47.9 and 79% for the alfalfa cubes and the concentrate portions of the diet, respectively, it can be calculated that a diet containing 30% forage and 70% concentrate, upon which the NRC (1989) protein requirements are based, would have had a pre-cecal protein digestibility of 69.7%. Thus the higher forage diets (60% forage and 40% concentrate) used by Coleman et al. (1997) and reported in Table 7-3 would have had pre-cecal protein digestibilities of between 76 and 86% that of the diet recommended by NRC (1989). Although no growth response due to supplemental protein and lysine was observed because the protein contents of the diets were quite high, it is clear that the proportion of protein digested in the small intestine can be markedly influenced by the diet. Thus equine nutritionists must give consideration to balancing rations on the basis of pre-cecal availability of protein and amino acids. This has become an accepted practice in swine nutrition (Sauer and Ozimek 1986) and is of even greater importance in equine nutrition since ileal digestibilities will vary more because of the higher proportion of forage used in equine diets.

Pre-cecal lysine apparent digestibility with the alfalfa cube diet was 48.1% (Table 7-3) When the mixed diets were fed the apparent digestibilities increased (P=.0001) from 51.1 to 62.3% with increased lysine intakes. The pre-cecal lysine digestibility in the concentrate portion of the diet can be calculated on the basis of the digestibility of lysine in the alfalfa cube diet and the proportion of alfalfa cubes in the 19% CP diet since the lysine concentration of the two diets are similar. Using this methodology the estimated pre-cecal digestibility of lysine in the concentrate diet was 83.5%, which means that in the small intestine the lysine in the alfalfa cubes was only about 60% as digestible as the lysine in the concentrates. There are no previously reported values for pre-cecal lysine digestibility in the horse. In the early weaned pig, the ileal digestibility of lysine for soybean meal supplemented diet was 76.2% (Fan et al. 1995) while in canola meal and barley diets, the ileal digestibility ranged from 66.7 to 71.7% (Fan and Sauer 1995). The values from our experiment therefore indicates that the lysine in the concentrates may be even more available in the horse than in the pig or that endogenous lysine excretions are less. Crude protein and the lysine pre-cecal digestibilities in the alfalfa cubes were similar in our study which is different than reported with pigs where Fan et al. (1994) noted that the dietary amino acid level may affect the apparent ileal digestibility independent of the dietary CP content.

Post-ileal Digestibility

Post-ileal digestion coefficients were determined as the difference between DM and nutrients presented to the cecum and fecal output.

Post-ileal DM digestibilities were 48.0, 50.4, 51.2, and 44.8% (P=.08) for the alfalfa cube, 15% CP, 17% CP and 19% CP diets, respectively. Other researchers have reported a range of DM digestibilities in the hind gut with all-forage diets of 37.2% (Hintz et al. 1971) and 41 and 48% (Gibbs et al. 1988). Large variations in post-ileal DM digestibility have been noted by Texas researchers when forage:concentrate mixed diets have been fed. Householder (1978) reported a post-ileal digestibility of 17.8% with 50:50 forage:concentrate diets, while Haley (1981) reported a similar digestibility of 15% with similar diets. In contrast, Brown (1987) reported a digestibility of 62%, similar to Gibbs (1982), who reported a 61.1% DM digestibility with diets containing 30 and 25% forage, respectively. It appears that, while a significant amount of dry matter is digested pre-cecally, the post-ileal dry matter digestion makes a major contribution to total dry matter digestion, particularly with high forage diets.

The post-ileal ADF digestibilities were 36.3,29.9,35.3 and 32.7% for the alfalfa cube, 15% CP, 17% CP and 19% CP diets (P>.05), respectively. The comparable NDF digestibilities were 32.6, 31.7, 37.2 and 30.4% (P>.05). Hintz et al. (1971) noted that the major site of digestion of NDF was the cecum and colon. With diets made up of 100% 60:40 20:80 forage:concentrate ratios, post-ileal NDF digestibilities were 29.2, 42.2, and 38.4%, respectively in their study. These results were similar to ours in that diet composition did not affect fiber digestibility, and that the hind gut appears to play a significant role in fiber digestion.

The crude protein digestibilities in the hind gut were 47.0,46.0,42.8 and 45.3% for the alfalfa cube, 15% CP, 17% CP and 19% CP diets, respectively. Comparable lysine digestibilities were 46.8,46.8,39.9 and 45.7%. There were no differences (P>.05) due to dietary treatment for either the CP or the lysine digestibilities. Hintz et al. (1971) reported a post-ileal protein digestibility of 29% with pelleted alfalfa, while Gibbs (1982) reported post-ileal N digestibilities of 65.66% and 66.8% for the two alfalfa hays. Our results are therefore intermediate as might be expected because of the different procedures used by these two other researchers. With diets that had 60:40 and 20:80 forage concentrate ratios Hintz et al. (1971) reported values of 35% for post-ileal disappearance of protein from the hind gut. Other results are available from studies with cannulated ponies as given below, however these results must be viewed with caution because of the sample collection procedure used as noted above. Brown (1987), who noted a lower pre-cecal protein digestibility (24.1%), reported a post-ileal protein digestibility of 58.7%. This would suggest that there was some level of compensation in the hind gut to utilize the protein presented to it. Gibbs (1982) reported that 71% of the nitrogen presented to the hind gut was digested with diets having 25:75 forage:concentrate ratios. These values plus those reported from this experiment demonstrate that there is potential for significant amounts of dietary nitrogen to be digested in the hind gut.

There are no reported values for post-ileal lysine digestion in the horse. While there was a disappearance of lysine in the hind gut it would be reasonable to assume this was due to microbial use of the lysine not the absorption of lysine from the hind gut. Bochröder et al. (1994) reported that in *in vitro* studies there was no transport of lysine, histidine or arginine from the mucosal to the serosal side of mucosa from the equine colon. Udén et al. (1982) also noted that because the fermentative digestion occurs after the enzymatic digestion there is limited opportunity for microbial end products to be used by the horse. The nitrogen disappearance is probably as ammonia which could contribute to the nonessential amino acid pool but provide little towards essential amino acid requirements of the horse.

True Digestibilities of Protein and Lysine

Estimates of true digestibility (slope) and metabolic fecal protein and lysine in the feces (x-axis intercept) were made for the forage:concentrate diets using regression analysis (Table 7-4).

True digestibility of protein in the total tract for the mixed diets was estimated at 90% with the daily output of metabolic fecal protein estimated at .34 g/kg BW. Since DM intakes were 1.3% of body weight, this corresponds to 2.6% of the DM intake. This total tract true digestibility value is similar to the 80 to 100% that reported by Potter et al. (1992) for a variety of mixed diets. NRC (1989) reported a true total tract digestibility of protein of 95% with a metabolic fecal protein of 4.2 % of DM intake for mixed alfalfa:concentrate diets thus their estimates of true digestibility and metabolic fecal protein are higher than ours. The lower metabolic fecal protein in our experiment may be a result of lower endogenous nitrogen due to a lower fiber content in the alfalfa used in our study. Level of dietary fiber has been shown in pigs to influence endogenous nitrogen secretion (Sauer and Ozimek 1986; Schulze et al. 1994).

Pre-cecal true protein digestibility was estimated at 89% with an estimated endogenous daily pre-cecal protein output of .80 g/kg BW. Potter et al. (1992) reported true digestibility for pre-cecal protein to range from 56 to 72% depending on diet composition, but again these estimates are dependent upon the validity of the ileal flow measurements.

True post-ileal protein digestibility in our experiment was estimated at 72% with a daily post-ileal endogenous protein output of .29 g/kg BW. Post-ileal true protein digestibility in the mixed diets was 72% which is lower than the 80 to 100% reported by Potter et al. (1992) for a series of experiments using mixed forage concentrate diets. The difference in these values is probably related to the comparable ileal digestibilities; if more protein is digested in the small intestine, less is available for the microbial population in the hind gut. Although the values from this experiment would suggest that significant amounts of protein are digested in the large intestine, the small intestine appears to be the primary site of protein digestion when diets containing 40% concentrate are fed.

Lysine true digestibility in the total tract was estimated at 97% with a daily estimated fecal lysine loss of 20.5 mg/kg BW. With DM intakes of 1.3% BW, this corresponds to a metabolic fecal loss of 0.19% DMI. The true digestibility of lysine pre-cecally was estimated at 98%

with a daily endogenous production of 39.3 mg/kg BW. Post-ileal lysine true digestibility was 88% and the daily endogenous lysine was estimated at 17.9 mg/kg BW.

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Table 7-1. Nutrient analysis and ingredient composition of the alfalfa cubes and concentrate mixes

Item	Alfalfa Cubes	% CP in Mixed Diets			SEM[a]	P[b]
		15	17	19		
Ingredients (% as-fed)						
Oats	---	38.6	38.2	38.1		
Barley	---	53.2	43.8	32.3		
Soybean Meal	---	---	10.5	22.9		
Canola Oil	---	5.8	5.3	4.8		
Mineral/Vit Premix[g]	---	2.4	2.2	1.9		
Composition of DM						
Dry Matter, %	90.7[c]	92.5[d]	92.5[d]	92.6[d]	0.41	0.007
Gross Energy, Mcal/kg	3.9[c]	4.25[d]	4.33[d]	4.29[d]	0.02	0.0001
Protein, %	19.0[c]	12.1[d]	15.7	18.1[f]	0.23	0.0001
Lysine, %	0.77[c]	0.39[d]	0.6	0.78[c]	0.012	0.0001
Acid Detergent Fiber, %	28.0[c]	10.9[d]	10.0[d]	9.5[d]	0.35	0.0001
Neutral Detergent Fiber, %	37.0[c]	24.2[d]	21.8[d]	21.7[d]	0.57	0.0001
Calcium, %	1.9[c]	0.58[d]	0.53[de]	0.47	0.03	0.0001
Phosphorus, %	0.22[c]	0.69[d]	0.72	0.63[f]	0.005	0.0001

[a] Standard error of the mean is based on four samples per mean.
[b] Probability
[g] Mineral/Vitamin Premix composed of 31% salt, 65% calcium phosphate, and 4% vitamin ADE premix (10,000,000 1,000,000 and 10,000 IU of vitamin A, D, and E, respectively).
[cdef] Within a row means lacking a common superscript are different ($P<.05$).

Table 7-2. Least square means for daily intake (g/kg body weight) of dry matter, protein, lysine, acid detergent fiber and neutral detergent fiber

	Alfalfa Cubes	% CP in Mixed Diets			SEM[a]	Probability
		15	17	19		
Number of Animals	4	3	4	3		
Body Weight, kg	257	257	257	257	19.05	
Dry matter, g	16	13.23	13.5	13.18	0.68	0.06
Crude protein, g	3.06^{b}	2.14^{d}	2.40^{c}	2.46^{c}	0.084	0.02
Lysine, g	0.124^{b}	$.082^{d}$	$.094^{c}$	$.102^{c}$	0.004	0.02
Acid detergent fiber, g	4.52^{b}	2.8^{c}	2.81^{c}	2.72^{c}	0.1	0.005
Neutral detergent fiber, g	5.99^{b}	4.21^{c}	4.19^{c}	4.05^{c}	0.14	0.008

[a] Standard error of mean
[bcd] Within a row means lacking a common superscript are different ($P<.05$).

Table 7-3. Total tract, pre-cecal, and post-ileal apparent digestibility coefficients (%) for dry matter, crude protein, lysine, acid detergent fiber and neutral detergent fiber

	Alfalfa Cubes	% CP in Mixed Diets			SEM[a]	P
		15	17	19		
Dry Matter						
Total Tract, % of intake	60.2^{e}	68.1^{c}	67.8^{cd}	64.3^{d}	1.18	0.001
Pre-cecal, % of intake	23.4^{c}	35.7^{d}	33.9^{d}	35.4^{d}	1.07	0.0001
Post-ileal, % of ileal flow	48.0	50.4	51.2	44.8	1.63	0.08
Acid Detergent Fiber						
Total Tract, % of intake	41.5	38.0	41.4	36.8	3.95	0.76
Pre-cecal, % of intake	7.4	11.8	8.94	6.2	2.7	0.52
Post-ileal, % of ileal flow	36.3	29.9	35.5	32.7	4.1	0.64
Neutral Detergent Fiber						
Total Tract, % of intake	43.48	43.8	43.8	39.6	3.36	0.68
Pre-cecal, % of intake	16.02	17.5	10.5	13.4	2.36	0.17
Post-ileal, % of ileal flow	32.6	31.7	37.2	30.4	2.97	0.35
Protein						
Total Tract, % of intake	71.9^{c}	73.4^{c}	75.3^{cd}	77.6^{d}	1.29	0.04
Pre-cecal, % of intake	47.9^{f}	51.9^{c}	55.6^{d}	60.0^{e}	0.8	0.0001
Post-ileal, % of ileal flow	47.0	46.0	42.8	45.3	3.04	0.72
Lysine						
Total Tract, % of intake	71.6	73.0	74.5	78.7	1.89	0.09
Pre-cecal, % of intake	48.1^{f}	51.1^{c}	55.9^{d}	62.3^{e}	0.91	0.0001
Post-ileal, % of ileal flow	46.8	46.8	39.9	45.7	4.71	0.54

[a] Standard error of the mean is based on 4,3,4,3 samples per mean for alfalfa cubes, 15%, 17% and 19%, respectively.

[cdef] Within a row means lacking a common superscript are different (P<.05).

Table. 7-4 Regression equations of protein and lysine digested (g/kg BW); vs daily intake (g/kg BW) for estimation of total tract, pre-cecal, and post-ileal true protein and lysine digestibility (g/kg BW) and endogenous and metabolic excretions (g/kg BW).

	Number of observations	Equation	R^2	SE[a]	P[b]	P[c]
Protein						
Total Tract	10	Y=-.34 + .90x	0.94	0.005	0.09	<.01
Pre-cecal	10	Y=-.80 + .89x	0.72	0.003	0.11	0.002
Post-ileal	10	Y=-.29 + .72x	0.85	0.003	0.03	<.01
Lysine						
Total Tract	10	Y=-20.5 + .97x	0.96	1.28	0.01	<.01
Pre-cecal	10	Y=-39.3 + .98x	0.79	8.92	0.05	<.01
Post-ileal	10	Y=-17.9 + .88x	0.85	1.17	0.01	<.01

[a] Standard error of the estimate of the regression equation (n=10)
[b] Probabilities of significance for the intercepts of the regression equations.
[c] Probabilities of significance for the slopes of the regression equations.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

The forage in a horse's diet can provide a significant portion of the animal's daily nutrient requirements. For a mature horse with minimal daily requirements for protein and lysine, a high quality forage such as alfalfa could supply the daily requirement for protein and lysine. However for young growing horses the recommendation is that diets should contain 30% forage and the remainder concentrate (NRC 1989) even though higher levels of forage can be used and the diets will supposedly meet the animals requirement for protein and lysine. Moreover, because of the reports of low pre-cecal protein availability, it is suggested that diets for young growing horses should contain highly digestible sources of protein and amino acids to ensure nutrient requirements are met. Therefore a series of experiments were conducted to determine the effects of protein supplementation for the suckling foal and the effect of protein concentration and level of forage on growth and nutrient availability in the horse.

The provision of supplemental nutrients in the form of a creep ration to suckling foals resulted in a 10% improvement in daily gain (Chapter 2). Protein level in the creep feed (13% vs 17%) had no influence ($P>.05$) on daily gain. Daily intakes of the creep ration were .85 and .54 kg/head ($P>.05$) for the 13 and the 17% CP diets, respectively. In addition to improved daily gains the creep-fed foals had improved (P<.0001) general assessment scores. The improved general assessment score was a significant factor in the sale price of the foals; prices for creep-fed foals averaged $940, while the non creep-fed foals averaged $384. There was no effect on any of the parameters measured due to the level of protein in the creep ration however the intake of the high protein creep feed was lower than anticipated. It is difficult to determine what factors would be responsible for the low intake. Therefore, further research into the factors that affect intake of creep ration for extensively managed foals is needed. In addition, because of the demonstrated improvement in sale prices of the creep-fed foals, it is obvious that the industry will be moving towards increased use of creep feeding. The effect of this practice on developmental orthopedic disease should receive further study.

The use of high forage diets (60% alfalfa cubes) resulted in acceptable daily gains .72 kg/d in a feeding trail with weanling fillies (Chapter 3). There were no differences (P>.05) in gains or feed:gain ratios due to dietary treatment when the diets contained 15,17 and 19% protein. These results, and those of Wall et al. (1998) with yearlings fed diets containing either alfalfa or soybean meal, suggest that alfalfa can supply a significant portion of the protein requirements for growing horses.

Ponies cannulated in the distal ileum were used to determine partial and total tract nutrient digestibilities. While numerous reports, as cited in Chapter 4, discuss techniques for installing cannulae they do not provide information on maintaining cannulated ponies or on the use of a larger cannula. In our experiences the removal of an external washer after the stoma has formed and reducing the length of the exteriorized portion of the cannula appeared to reduce animal discomfort. When these procedures were used there was also a reduction in problems with cannulas being dislodged. The use of a cannula with an internal diameter (2.5 cm) larger than used by other researchers allowed for easy sample collection and consistent samples, especially with high forage diets. The consistency of samples collected from these cannulae is reflected in the similarity of DM digestibilities as determined by the dual marker versus single marker techniques (see Chapter 6).

External markers have been used in equine digestion studies for many years however chromium mordanted hay fiber and Co-EDTA have not been widely used. The use of either marker gave comparable total tract DM digestibilities to values determined using the total fecal collection method as discussed in Chapter 5. Although it was found that there was no difference in DM digestibility determined by total collection or Cr-hay fiber methods for diets containing 60% forage and 40% concentrate, the percentage recovery of marker was lower with low forage diets and increased curvilinearly (R^2 =.85) as the percentage forage in the diet increased to 50% of the diet. Recovery of the fluid marker Co-EDTA was variable. It was concluded that Cr-hay fiber is a useful marker for horse digestion studies, however Co-EDTA gave more variable results due in part to a difficulty in obtaining consistent daily intakes of this marker. Regardless of marker selected, total recoveries were not always obtained. This lack of total recovery can affect estimation of fecal output or digesta flow therefore researchers should correct marker intakes based on the fecal recoveries, especially when diets contain less than 60% forage as discussed in Chapter 5.

The representativeness of samples obtained from the ileum, and hence the use of single markers for digesta flow has not been studied in the horse. In Chapter 6 the determination of ileal DM digestibilities were based on either a single marker or the dual marker technique of Farchney (1975). The dual marker technique is used to correct potential problems with unrepresentative samples and allows the composition of true digesta to be determined mathematically based on the marker concentrations in the two phases of the digesta. We found no differences (P>.05) in DM digestibility as determined by either a single marker method or the dual marker technique. This suggests that the sampling procedure of allowing the first bit of digesta to escape and then collecting ileal samples for 4 min provided representative samples. Titgemeyer (1997) has reported that if a representative sample was collected then a single marker would be suitable. It is not enough, however to obtain a representative sample of ileal digesta at any one time, the samples taken must be representative of the total daily flow. All other researchers who have used cannulated animals have used a sampling protocol where samples are taken more frequently around peak digesta flow post-feeding, followed by less frequent sampling for the remainder of the inter-feeding period,or have not sampled for the total day (see Chapter 7). Sampling frequently at peak flow can result in lower concentrations of marker in the sample DM than occurs at other times which would result in an overestimation of DM flow. In contrast, if more samples are taken when marker concentration in ileal DM is higher than the mean, there could be an underestimation of DM flow. In either situation the sampling procedure will greatly affect results. It therefore appears as if all previous results with equines in which ileal flow has been measured with cannulated ponies are suspect. In contrast the use of the sampling protocol involving collecting samples over 4 min intervals for each h of the day as we have done (see Chapters 5, 6, and 7) appeared to provide a representative sample of digesta and marker concentration. This protocol follows that suggested by Owens and Hanson (1992). We believe that we have obtained the first valid measurement of ileal flow in cannulated ponies.

There were no differences (P>.05) in total tract or partial tract ADF or NDF digestibilities in our ponies. Similar results appear in the literature for total tract ADF digestibility which may have implications for prediction of digestibility of forages for horses. The pre-cecal ADF digestibilities ranged form 6.2 to 11.8% while NDF values were 10.5 to 16.0%. As horses do not produce enzymes to digest fiber, some of the fiber disappearance must be due to bacterial fermentation in the small intestine. Kern et al. (1974) reported that there was a bacterial population in the small intestine of the horse. The hind gut of the horse is, however, the major site of fermentative digestion and therefore the major site of fiber digestion in the horse.

Total tract protein digestibilities were 71.9, 73.4, 75.3 and 77.6% (P=.04) for the alfalfa cube 15% CP, 17% CP and the 19% CP diets, respectively. The values for the alfalfa cubes was similar to literature values (Chapter 7). For the mixed diets protein digestibility tended to increase with increasing protein in the diet which may be due to a diluting effect on metabolic fecal nitrogen as reported by Slade and Robinson (1970). Total tract lysine digestibilities for the mixed diets showed a similar trend as for protein; as dietary intakes increased digestibility increased. There are no previous reports in the literature for lysine digestibility in the horse. The total tract lysine digestibilities for the mixed diets were slightly below the values reported for all-concentrate diets containing soybean meal fed to early weaned pigs (Fan et al. 1995).

Pre-cecal protein digestion for the mixed diets was 55% and increased (P=.001) with increasing dietary protein concentration. The all-forage diet had a pre-cecal digestibility of 47.8% which is slightly higher than the 41.1% reported by Hintz et al. (1971) on the basis of a slaughter technique and much higher than the 1.3 and 21% reported by Gibbs et al. (1988) who used ileal cannulated ponies. While the quality of forage could be a factor in these differing digestibilities, as discussed previously the method of sampling could account for much of this variation.

Pre-cecal lysine digestibilities have not been reported for the horse. With the mixed diets pre-cecal lysine digestibilities increased (P=.0001) with increasing dietary levels.

Pre-cecal protein digestibilities ranged from 52 to 60% for the 60% forage and 40% concentrate diets. From these values and the pre-cecal digestibility of 47.8% for the forage, the pre-cecal protein digestibility in concentrate was calculated as 80%. Hence it follows that the protein in the alfalfa cubes was only 60% as digestible pre-cecally as that in the concentrate. This reduced digestibility is in agreement with our original hypothesis. Since there are differences in the pre-cecal digestibilities between forages and concentrates, and since protein digested after the ileum is not available to the horse (Bochröder et al.1994), it is apparent that equine diets must be formulated and evaluated on the basis of pre-cecal protein and amino acids availabilities. However requirements for this are not known and research is required in this area. Also future research needs to concentrate on the determination of factors influencing amino acid availabilities in the small intestine of the horse. Use of the mobile nylon bag technique may be possible for this purpose (Sauer and de Lange 1992; Hyslop and Cuddeford 1996). Eventually, though, we will need to increase our understanding of the factors influencing endogenous intestinal losses of nitrogen and amino acids in the horse.

A. Conclusions

The following conclusions can be drawn.

1) Creep feeding extensively managed foals can improve growth rates and overall condition at weaning which can enhance marketability. Protein in the creep ration above 13% did not affect the parameters measured when the protein content of the pasture forage was 10%.

2) The use of higher levels of forage(> 50%) in the diets of young growing horses can provide adequate intakes of protein and lysine for growth rates up to 0.70 kg/day.

3) The sampling protocol with cannulated animals must ensure that representative samples are taken. Samples should be taken at regular intervals thoughout the day to represent the entire 24 h period.

4) The pre-cecal digestibility of protein and lysine is affected by diet composition. Protein and lysine in high quality alfalfa cubes are 60% as digestible as the protein and lysine in a soybean meal supplemented concentrate.

5) Pre-cecal protein digestibility of feeds should be considered when formulating diets for horses, particularly as it applies to the forage component of the diet.

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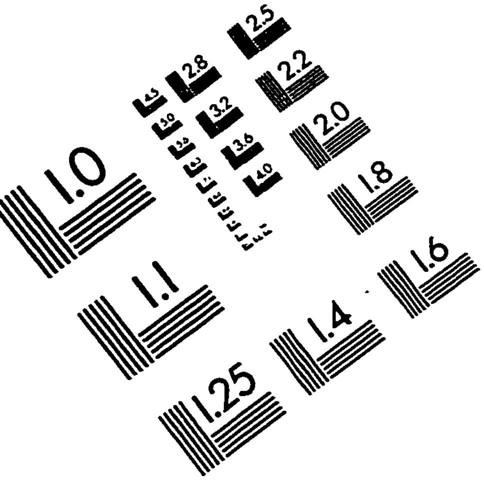
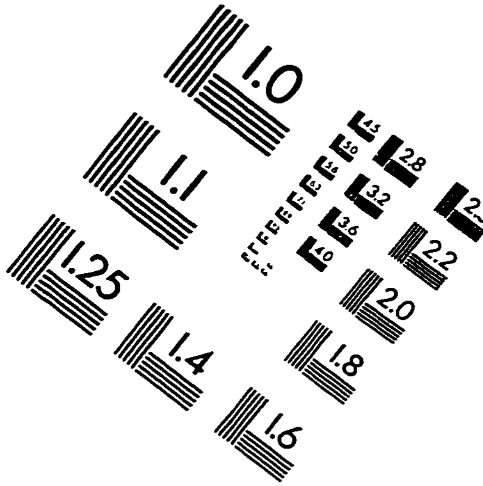
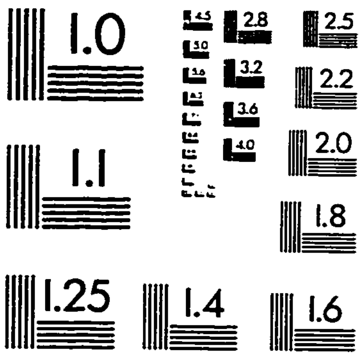
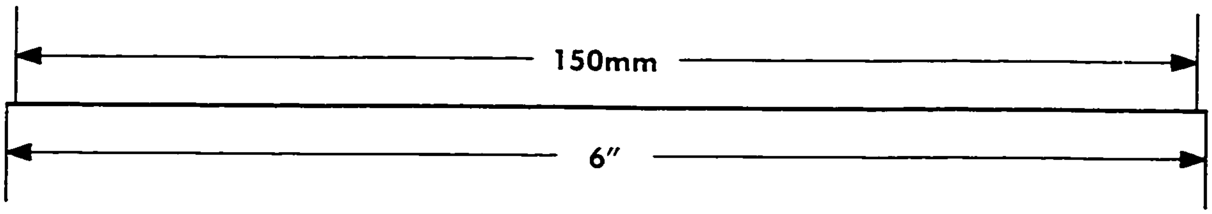
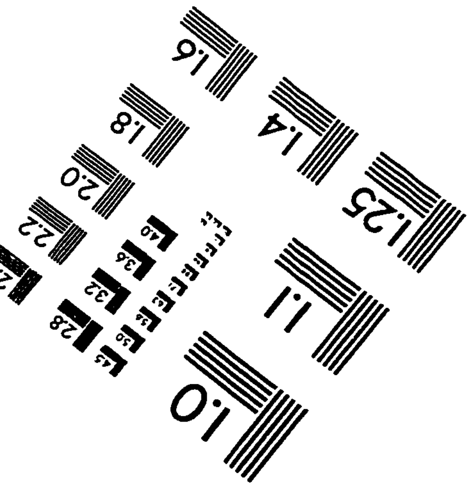
IMAGE EVALUATION TEST TARGET (QA-3)





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