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Effects of feeding fat on nutrient digestion in cannulated ponies fed a forage diet

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Abstract

Horse diets are often supplemented with dietary fat to increase caloric intake, and although the effects of fat on fiber digestibility have been studied, this research has not included the use of cannulated subjects. A decrease in fiber digestibility due to fat would be detrimental to equine performance, and thus, the current study utilized cannulated ponies as an equine model for any effects on fiber digestibility due to supplemental dietary fat on a forage diet. Five Shetland/hackney pony mares with an ileal and cecal cannula were fed hay, 2 kg/d alfalfa pellets and vegetable oil at 0, 5, 10 or 15% of total diet. Experimental diets were fed twice a day at 0600 and 1600 hours. Ponies were allowed a 14-d diet adjustment period, after which ileal and cecal cannula samples and fecal grab samples were collected at 1, 3 and 5 d post diet adjustment. On day 1, samples were taken at 0, 90, 180, 270 min and 7 and 10 h. Day 3 samples were taken at 30, 120, and 210 min, and 5 and 8 h. On d 5 samples were taken at 60, 150, and 240 min and 6 and 9 h post feeding. Samples were analyzed for crude protein, fat, acid-detergent fiber, acid-detergent lignin, and apparent digestibility of dry matter, crude protein, fat, and acid-detergent fiber. No effects were found on fiber digestibility due to fat supplementation up to 15% of the total diet in a pony model ($P > 0.1$). Results indicated that equine diets could be supplemented with up to 15% of fat in the form of vegetable oil to increase digestible energy with no negative impact on fiber digestibility.

Introduction

Providing supplemental fat has become popular in horse diets due to growing research in this area demonstrating benefits to performance, and yet, the benefits to this type of supplementation have come to question by conflicting studies over the years. Jansen *et al.* [1] reported that amount of dietary fat negatively affected digestibility of crude fiber, neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and nitrogen-free extract. However, several earlier studies [2-4] examined the effects of corn oil, up to 15% of the total diet, finding no effect on digestibility of fiber. Nevertheless, a later study by Delobel *et al.* [5] reported the inclusion of fat at 8 % had a positive effect on NDF.

The question to the benefits of fat supplementation may, in part, be related to the amount of fat being supplemented to a diet. The common performance horse diet typically has a fat supplementation below 15%. This practice has been supported by research that fed to horses additional fat in the diet at 15 to 28% [6]. A negative effect on digestibility of fiber was reported when the amount of fat possibly exceeded the lipolytic capacity in the ileum. This same research reported a decreased digestibility of fiber in the cecum due to fat coating of fiber particles. Furthermore, Meyer *et al.* [7] and Jansen *et al.* [6] reported a decrease in crude protein (CP) digestibility when dietary fat was increased. While a relationship with the amount of fat supplementation and digestibility has been shown, thorough insight into digestive activity in the ileum and cecum as related to this type of diet has yet to be explored using a dual cannulation model [8].

Previous research utilized some starch in diets with additional fat, and this type of diet may be a confounding factor in determining fiber digestibility as related to fat supplementation. In contrast to other studies reporting a negative digestibility, in a diet of mixed hay and concentrate, addition of fat in the form of soy oil, up to 15% of dry matter (DM), was reported by Zeyner *et al.* [9] to increase fiber digestibility. Nevertheless, current studies have lacked a pure forage diet in research focused on fat supplementation. Therefore, the objective of the current study was to determine effects of feeding fat, at differing amounts from 0 to 15%, on digestibility of nutrients to a forage diet utilizing dual cannulated ponies.

Material and methods

Animals and diets

Five Shetland/Hackney cross dual (ileal and cecal) cannulated pony mares, aged 2 to 10 years of age, were utilized for the study with bodyweight (BW) ranging from 156 to 199 kg. Ponies were fed and housed in individual stalls within the same barn at the equine unit of the Leveck Animal Research Center of the Mississippi Agriculture and Forestry Experiment Station at Mississippi State University. Ponies were hand walked for 45 min / d for exercise during the study. All animal procedures were approved by the Mississippi State University Institution Animal Care and Use Committee.

Diets consisted of ad libitum access to 80% Bermudagrass (Paspalumdilatatum) and 20% Dallisgrass (Cynodondactylon) hay

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(Table 1), a trace mineral salt block (Table 2), and water throughout the study. During the 4 trial periods 2 kg/d as fed of alfalfa pellets (Pride and Pleasure, Faithway Guntersville, AL) were provided to all ponies. Alfalfa pellets acted as a carrier for oil consumption to ponies at differing fat amounts. Vegetable oil was provided at 0, 5, 10, or 15% of total diet, fatty acid composition (Table 3). Once hay and pellet intakes were determined by assuming a 1.2% BW DM intake of hay, supplemental fat was adjusted as a percent of the total diet. Hay, pellets, and oil offered/ refusals, and thus intake, were measured daily during the entire experiment (Table 4-5).

Ponies were randomly assigned to each treatment so that each pony was given 1 of 4 diets during each period. Each 5-day trial period, with a 3-day sample collection, was separated by a 14-day period where the ponies were adjusted slowly to the new diet. The four experimental diets consisted of ad libitum access to 80% Bermuda (*Paspalumdilatatum*) / 20% Dallisgrass (*Cynodondactylon*) hay paired with alfalfa pellets coated with vegetable oil (0, 5, 10, or 15% added fat) fed twice daily (0600 and 1600). Three additional experimental periods were implemented such that every animal randomly received each of the 4 diets.

Table 1. Nutrient composition of hay, alfalfa pellets and oil fed to ponies.

Offered	DM (%)	CP (% DM)	NDF (% DM)	ADF (% DM)	EE (% DM)
Hay	84.46	10.52	79.40	33.85	1.21
Alfalfa Pellets	89.23	16.39	48.52	32.08	10.17
Oil	89.61	0	0	0	100.00

Table 2. Trace mineral salt block nutrient composition provided to the ponies at ad libitum access.

Mineral	%
Sodium Chloride	98.236
Ferrous Carbonate	0.526
Zinc Oxide	0.486
Manganese Oxide	0.334
Iron Oxide	0.252
Cupric Sulfate	0.120
EthylenediamineDihydriodide	0.035
Mineral Oil	0.020
Cobalt Carbonate	0.011
Calcium Iodate	0.011
Artificial Flavor	0.005

Table 3. Fatty acids in vegetable oil provided to ponies on diets at 5, 10, and 15% added fat to diets (µg/mL and % of total fatty acids in oil).

Fatty Acids	µg/mL	%
C14:0	63.68	0.57
C14:1	3.23	0.03
C16:0	381.39	3.42
C16:1	150.47	1.35
C18:0	457.57	4.10
C18:1	1052.21	9.43
C18:2	930.60	8.34
C18:3	7000.38	62.77
C20:0	413.95	3.71
C20:1	428.93	3.85
C20:3	12.14	0.11
C20:4	36.73	0.33
C20:5	1.47	0.01
C22:1	7.82	0.07
C24:0	204.32	1.83
C24:1	7.91	0.07

Table 4. Hay and total dry matter intake (DMI) by 0, 5, 10, and 15% oil of the total diet (kg/d).

Variables	Diet (% fat)				
	0	5	10	15	s.e.m.
Hay DMI, kg/d	3.00	2.42	2.43	2.35	1.081
Total DMI, kg/d	5.04	4.78	4.81	4.72	0.683
s.e.m.	0.633	1.081	1.010	0.755	

Table 5. Hay and total diet dry matter intake (DMI) for 0, 5, 10, and 15% oil of the diet (% BW/d).

Variables	Diet (% fat)				
	0	5	10	15	s.e.m.
Hay DMI, % BW /d	1.59	1.32	1.31	1.29	1.081
Total DMI, % BW /d	2.66	2.63	2.63	2.60	0.683
s.e.m.	0.633	1.081	1.010	0.755	

Ileal and cecal samples were collected via cannula during the 5-day trial period. Samples on d 1 were collected at 0, 90, 180, and 270 min and h 7 and 10 post 0600 feeding of supplement. Collections on d 3 were obtained at 30, 120, and 210 min and 5 and 8 h post feeding. Lastly, collections on d 5 occurred at 60, 150, 240 min and 6 and 9 h post feeding such that samples for each week were obtained at 0, 30, 60, 90, 120, 150, 180, 210, 240, and 270 min. Fecal samples were hand collected immediately after defecation on d 3 and 5 during the sampling period. Ileal, cecal, and fecal samples were stored at -20°C. Hay, pellets, and oil samples were collected over each trial period. Hay, oil, pellets, ileal, cecal, and fecal samples were dried for 36 h in a 70°C forced air oven, and then, ground through a 2 mm screen with a Wiley Mill (model 4, Thomas Wiley).

Laboratory analysis

Crude protein was determined by Kjeldahl-N technique [10] and CP calculated by nitrogen (%) X 6.25. Fat by ether extract (EE) was determined by standard Association of Official Analytical Chemists (AOAC) methodology [10]. Acid-detergent fiber, NDF, and acid-detergent lignin (ADL) were determined according to Van Soest *et al.* [11] procedures.

Apparent digestibility of DM, CP, EE, NDF, and ADF were analyzed using ADL as an internal marker. Digestibility was determined by the following equation:

Digestibility = 100-100 (% ADL of diet consumed / % ADL of location) X (% nutrient in Location / % nutrients in diet consumed).

Statistics

After laboratory analysis of obtained samples, data was pooled into "common" time references due to the inconsistencies in number of samples (ileal, cecal, and fecal). Time references were hourly for apparent ileal and cecal digestibility (0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 h post feeding of alfalfa pellets and oil). For apparent total tract digestibilities, these time references were every 1.5 h post feeding of alfalfa pellets and oil (0, 1.5, 3, 4.5, 6, 7.5, and 9 h).

The current study was analyzed based on an incomplete randomized block design. Ponies were designated as the block; treatment was considered an effect; and repeated measure was the collection time (reference period, described above). Trial period was not significant, and thus, was removed from the model. Data were analyzed according to the mixed model for repeated measures (V. 9.1, SAS Inst., Inc., Cary,

NC). Means were separated when significant ($P < 0.1$) using least significant differences.

Results

Two ponies required substitution for individual trial periods as a result of increased supplemental fat at 15% of the diet. Ponies removed exhibited weight loss due to a lack of feed intake for more than 3 d. Lethargy, anorexia, or limited feed refusal was observed for all ponies fed 15% fat diet, but only the two ponies were considered to demonstrate significant weight loss that could be detrimental to overall health.

Apparent ileal CP, NDF, or ADF digestibility was not affected ($P > 0.1$) by treatment or time (Table 6-7). There was a treatment by time interaction ($P < 0.1$) for apparent ileal fat digestibility (Table 8). For the 0% fat diet, from h 0 to 1 post feeding, a decrease in apparent ileal fat digestibility from 61.09 to 41.06% occurred, and then, was consistent until 7 h post feeding when apparent digestibility increased to 132.16%. From 7 to 8 h post feeding, apparent ileal digestibility of fat decreased to 34.2%, and then, increased to 78.86% by 9 h post feeding. Diets with additional fat (5, 10, and 15%) had greater apparent ileal digestion of fat compared to no additional fat, but were not affected by time ($P > 0.1$).

Apparent cecal digestibility of fiber, NDF, and ADF were not significantly different ($P > 0.1$) among time or treatment (Table 9,10). Apparent cecal digestibility of CP decreased ($P < 0.1$) from 0 to 2 h post feeding followed by an increase in CP until 4 h post feeding. At 5 h post feeding, CP decreased by 9.96% followed by an increase at 6 and 7 h post feeding, then decreased from 8 to 9 h (Table 9). As expected, added dietary fat affected ($P < 0.1$) apparent cecal digestibility of fat from 0% fat at 19% apparent digestibility to 83% apparent digestibility at 5% and 10% added fat. Ponies fed 15% added dietary fat increased apparent digestibility to 89% (Table 11). Apparent total tract digestibility of NDF, ADF, and fat (Table 11) was affected by time ($P < 0.1$). Apparent total tract digestibility of NDF and ADF were not affected ($P > 0.1$) by added fat; however, adding fat (5, 10, and 15%) increased ($P < 0.1$) apparent total tract digestibility of fat (Table 12).

There was treatment by time interaction for apparent total tract protein digestibility (Table 13). At 0 h post feeding apparent total tract digestibility of protein was greatest ($P < 0.1$) compared to other time periods. Adding fat to the diet decreased apparent total tract protein digestibility at 0 h post feeding. However, at 3 h post feeding only 10% fat decreased protein digestibility compared to 0% fat. Also, 5 and 15% fat apparent total tract protein digestibility was an intermediate apparent CP digestibility compared to 0% and 10% added dietary fat.

Table 6. Apparent ileal protein, NDF and ADF digestibilities from 0 h before feeding throughout a 10 h collection period after alfalfa pellet consumption with different amounts of fat (0, 5, 10, or 15%).

Apparent Ileal Digestibility (as a % of total diet)			
Time (h post-feeding)	**Protein**	**NDF**	**ADF**
0	60.60	32.75	42.50
1	36.66	34.27	20.24
2	29.06	21.92	7.30
3	33.53	29.76	19.18
4	31.73	21.17	12.50
5	27.26	18.96	3.72
6	37.12	20.90	8.75
7	37.80	27.53	9.24
8	17.42	27.92	9.78
9	30.97	28.75	7.01
s.e.m.	20.021	9.643	11.632
Means within columns are not significantly different ($P > 0.1$).			

Table 7. Apparent ileal protein, NDF, and ADF digestibility for ponies fed different amounts of fat (0, 5, 10, or 15%).

Apparent Ileal Digestibility (as a % of total diet)			
Diet (% additional fat)	**Protein**	**NDF**	**ADF**
0	38.70	26.03	21.10
5	35.87	26.79	17.79
10	28.48	27.24	23.94
15	31.80	27.03	19.14
s.e.m.	11.064	5.981	12.042
Means within columns are not different ($P > 0.1$).			

Table 8. Apparent ileal EE digestibility as a % of total diet for all ponies between diet (% fat) and time post feeding.

	Diet (% fat)				
Time (h)	0	5	10	15	s.e.m.
0	$61.09^{b,y}$	91.23^{z}	94.49^{z}	95.05^{z}	7.710
1	$41.06^{a,y}$	91.98^{z}	86.46^{z}	91.46^{z}	6.473
2	$20.93^{a,y}$	86.48^{z}	93.26^{z}	93.89^{z}	10.922
3	$34.35^{a,y}$	91.95^{z}	93.01^{z}	95.01^{z}	7.750
4	$40.20^{a,y}$	90.81^{z}	93.83^{z}	97.32^{z}	6.461
5	$28.98^{a,y}$	88.09^{z}	92.79^{z}	91.51^{z}	10.930
6	$35.16^{a,y}$	94.04^{z}	88.55^{z}	97.37^{z}	10.923
7	$132.16^{c,z}$	90.99^{y}	84.99^{y}	92.88^{y}	10.924
8	$34.20^{a,y}$	94.44^{z}	95.23^{z}	95.03^{z}	10.920
9	$72.86^{b,y}$	85.07^{yz}	91.77^{yz}	97.83^{z}	10.921
s.e.m.	10.930	10.841	10.881	7.710	

$^{a, b, c}$Least squares means within columns with same superscript letters were not different ($P > 0.1$).
$^{y, z}$Least squares means within rows with same superscript letters were not different ($P > 0.1$).

Discussion

While fecal sampling is a common practice in evaluating effectiveness of digestive tract functioning, equine feces may not give us the best insight to activities of all aspects of the digestive tract [12]. Nevertheless, the use of cannulation studies in the field may not always be the most feasible approach to understanding the equine digestive tract. In this study, the approach did limit the number of subjects, but from the information obtained, comparisons can be made with previous studies using non-cannulated subjects to assist in supporting current results. In the present study, supplementation of vegetable oil did not affect digestibility of fiber. Similarly, Kane *et al.* [4], McCann *et al.* [13], Meyer *et al.* [14], Hughes *et al.* [15], and Bush *et al.* [3] reported no effect on fiber digestibility with the inclusion of up to 15% fat.

As expected, dietary fat had a significant effect on apparent EE digestibility in ileum, cecum and total tract. Bush *et al.* [3] reported the largest fat digestibility was observed when horses were fed 15% dietary fat, while treatments of 5% and 10% fat were not different from 0%. Delobel *et al.* [5] concluded that the addition of linseed oil increased fat digestibility by 26.4%. Estimates of apparent digestibility of fat by ponies were 42 to 49% for forage and 88 to 94% of supplemented fat and oil [16]. However, Meyer *et al.* [17] and Webb *et al.* [18] reported no effect of diet on fat digestibility when horses were fed similar fat amounts. Digestibility differences in previous studies could be due to overall diet composition, and thus, the use of a forage-based diet was selected for this study and recommendations are made for future studies that similar considerations are made in selection of diet composition.

Diet selection for this study included the type of fat used for supplementation. This study utilized a vegetable oil for fat supplementation. Vegetable oil, which is dense in triglycerides, has been documented to increase the proportion of digested true fat [5].

Increased dietary fat results in the increase of pre-ileal fat digestibility and of jejuno-ileal flow [7]. This lipolysis should be completed in the small intestine with no significant amount of fat entering the cecum to affect fiber digestibility [5]. Therefore, differences seen in results between this study and previous research may simply be due to the type of fat utilized.

Digestibility of CP was like previous results with comparable supplemental fat. Although ileal CP digestibility was not affected by added fat, a slight decrease in CP was observed at 4 to 5 and 7 to 8 h post feeding, which indicated a potential time effect in the cecum. Meyer *et al.* [7] and Jansen *et al.* [6] reported a decrease in CP with the inclusion of fat into the diet. Meyer *et al.* [7] hypothesized the large amount of fat decreased apparent digestibility in the small intestine because of the increased endogenous nitrogen flow related to the stimulation of digestive secretions. Although Delobel *et al.* [5] reported no effect on CP when vegetable oil was added to the diet, concentrate was also presented to the diet unlike the current study. Oil presented with concentrate could be less likely to coat fiber particles or could allow fat to be more digestible when compared to fiber only diet.

Table 9. Apparent cecal protein, NDF, ADF, and EE digestibility throughout a 10 h collection period for ponies fed different amounts of fat (0, 5, 10, or 15%).

Apparent Cecal Digestibility (as a % of total diet)				
Time (h post-feeding)	**Protein**	**NDF**	**ADF**	**EE**
0	33.27^{c}	27.75	13.08	70.06
1	27.84^{bc}	16.99	7.25	71.18
2	24.35^{ab}	19.15	11.24	66.38
3	27.35^{bc}	19.64	5.34	68.38
4	33.42^{c}	22.92	7.56	64.40
5	23.46^{ab}	23.72	7.89	69.57
6	26.96^{abc}	19.73	13.40	66.92
7	35.04^{c}	19.21	6.73	74.93
8	19.67^{a}	24.88	11.24	70.15
9	21.14^{ab}	17.36	6.01	66.87
s.e.m.	4.64	6.51	5.85	3.35
$^{a, b, c}$Least squares means within columns with same superscript letters were not different (P > 0.1).				

Table 10. Apparent cecal protein, NDF, ADF, and EE digestibility for all ponies fed different amounts of fat (0, 5, 10, or 15%).

Apparent Cecal Digestibility (as a % of total diet)				
Diet (% additional fat)	**Protein**	**NDF**	**ADF**	**EE**
0	31.74	16.17	4.68	19.10^{a}
5	30.69	23.34	11.40	83.02^{b}
10	28.72	21.57	5.48	83.17^{b}
15	22.21	22.59	11.71	89.01^{b}
s.e.m.	5.445	2.072	3.120	4.671
$^{a, b}$Least squares means within columns with same superscript letters were not different (P > 0.1).				

Table 11. Apparent total tract NDF, ADF, and EE digestibility throughout a 9 h collection period for all ponies fed different amounts of fat (0, 5, 10, or 15%).

Apparent Total Tract Digestibility (as a % of total diet)			
Time (h post-feeding)	**NDF**	**ADF**	**EE**
0	51.00	37.72	85.91
1.5	40.98	21.73	78.83
3	36.86	18.70	76.53
4.5	36.23	19.32	81.38
6	44.82	28.93	85.02
7.5	34.58	22.67	81.45
9	36.02	20.22	86.27
s.e.m.	8.720	12.613	6.201
Means within columns are not different (P > 0.1).			

Table 12. Apparent total tract NDF, ADF, and EE digestibility for all ponies fed different amounts of fat (0, 5, 10, or 15%).

Apparent Total Tract Digestibility (as a % of total diet)			
Time (h post-feeding)	**NDF**	**ADF**	**EE**
0	51.00	37.72	85.91
1.5	40.98	21.73	78.83
3	36.86	18.70	76.53
4.5	36.23	19.32	81.38
6	44.82	28.93	85.02
7.5	34.58	22.67	81.45
9	36.02	20.22	86.27
s.e.m.	8.720	12.613	6.201
Means within columns are not different (P > 0.1).			

Table 13. Apparent total tract CP digestibility displayed in 90 min increments from 0 to 10.5 h post feeding for all ponies.

	Diet (% fat)				
Time (1.5h)	0	5	10	15	s.e.m.
0	78.25^{b,z}	31.53^{y}	24.46^{y}	37.20^{y}	10.922
1.5	45.29^{a}	43.58	33.61	39.69	8.621
3	52.23^{a,z}	41.12^{yz}	35.44^{y}	41.77^{yz}	8.642
4.5	48.53^{a}	44.38	38.07	43.62	8.950
6	47.93^{a}	42.41	34.29	33.57	10.175
7.5	50.04^{a}	45.32	36.67	42.99	8.724
9	47.36^{a}	31.44	32.66		10.364
s.e.m.	10.170	10.361	10.151	10.960	
$^{a, b}$Least squares means within columns with same superscript letters were not different (P > 0.1). $^{y, z}$Least squares means within rows with same superscript letters were not different (P > 0.1).					

Jansen *et al.* [1] hypothesized increased lipid digestibility from the diet would increase lipid amounts in the large intestine, thus decrease total tract output of microbial nitrogen due to decreased cecal-colonic microbial growth. In the current study, approximately 95% of the dietary fat was digested before the ilium with minimal fat entering the cecum. Therefore, fat was determined not to effect microbial environment in the cecum because the amount entering the cecum is negligible.

Conclusion

Performance horses are often supplemented with dietary fat to increase caloric intake. However, previous effects of fat on fiber digestibility in equine have been conflicting. This may be due to differences in the dietary composition studied or in the lack of understanding of the functioning of the different aspects of the digestive tract without the use of multiple cannulation sampling. The current study using dual cannulations at the ileum and cecum found fat to not affect apparent fiber or CP digestibility when included up to 15% of a forage-based diet. Lack of additional fat effects, from 5 to 15%, means that performance horses can be fed vegetable oil for highly digestible energy. Furthermore, effects of fat at different inclusion rates on fiber digestibility and impacts of differing dietary nutrients could be the cause for the differing results from previous studies, and thus, these factors should be a consideration for further studies using dual cannulations.

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