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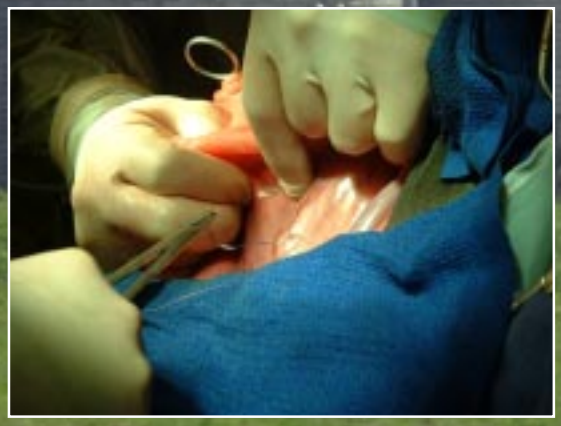

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BEEF RESEARCH REPORT

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Front cover "cells" photo: E. Schröck and T. Ried, NIH/NHGRI. Used with permission.

Contents

Interactions between Supplement Energy Source and Tall Fescue Hay Quality on Forage Utilization by Beef Steers

B.G. Fieser and E.S. Vanzant

Introduction

Energy supplements are often incorporated into diets of grazing animals to increase animal performance. According to NRC requirements and published reports using steers grazing tall fescue, metabolizable protein needs will generally be met by grazed fescue alone. Therefore, energy supplementation is commonly used to increase animal performance.

Energy supplements are often divided into two categories: starch- or fiber-based. Fiber-based supplements are generally by-product feedstuffs comprised of highly digestible fiber, such as soybean hulls, wheat bran, or beet pulp. Starch-based supplements generally consist of cereal grains such as corn, sorghum, or barley. Whereas starch-based supplements have been shown to depress forage digestion, fiber-based energy supplements generally do not have this negative effect.

Some research indicates that the negative effects of starch are increased with declining forage quality, suggesting that the benefits of fiber-based supplements may be greater with lower-quality forages. To our knowledge, no studies have been conducted to test this hypothesis directly. This study was undertaken to determine how corn and soybean hulls interact with different maturities of tall fescue and what implications this may have on supplementation strategies.

Procedures

Twelve ruminally cannulated, crossbred steers (initial BW 228 kg) were used in three simultaneous four-by-four Latin squares with a three-by-four factorial treatment arrangement. Each square represented a supplemental treatment. Steers were blocked by weight and assigned to one of three supplemental treatments:

- no supplement,
- pelleted soybean hulls, or
- coarsely cracked corn.

Supplements were fed once daily at 0.75% BW (on an as-fed basis), and tall fescue hay was offered daily at 150% of average intake. All steers, including those receiving no supplement, were offered 40 g of a commercial mineral mix at feeding time.

The second factor was tall fescue hay maturity:

- vegetative,
- boot stage,
- heading stage, and
- mature.

Hay was harvested at four different times during the 2000 grazing season from endophyte-infested tall fescue pastures adjacent to those being used for a concurrent grazing study in an attempt to provide four distinctly different hay maturities. Chemical composition of the four hay maturities and supplements is presented in Table 1.

Each of the four periods consisted of 21 days. Steers were adapted to diets for 12 days. Total feed intake and total fecal and urine output were measured and sampled on Days 13 to 19. A marker for measuring ruminal fluid dilution rate (Cr:EDTA) was administered intra-ruminally just prior to feeding on Day 20, and then ruminal fluid was sampled at three-hour intervals for 12 hours on Day 20 and once (24 hours after dosing) on Day 21. Supplement, hay, ort, and fecal samples were dried at 55°C in a forced-air oven to a constant weight and then ground in a Wiley mill to pass through a 1-mm screen. Aliquots of ruminal fluid were immediately measured for pH, acidified, and frozen for subsequent analysis of VFA and NH_3, or were frozen without additive for subsequent measurement of Cr concentrations.

Feed, orts, and fecal samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP; by gas N analysis), and neutral detergent fiber (NDF). Feed samples were also analyzed for acid detergent fiber (ADF) and acid detergent lignin (ADL). Urine samples were analyzed for N.

The MIXED procedure of SAS was used to evaluate all data using a model appropriate for a factorial treatment arrangement in a replicated Latin square design. Fermentation characteristics were analyzed as repeated measures. Protected ($P < 0.10$) Fisher's LSDs were used to separate treatment means.

Results and Discussion

Intake and digestibility

Supplement type x hay maturity interactions were not detected ($P > 0.10$) for hay (Figure 1), total (Figure 2), or digestible dry matter intake (DMI; Figure 3), which all decreased ($P < 0.01$) as hay maturity increased from vegetative through heading stages and increased slightly with the mature hay relative to the heading stage hay. Reasons for the higher intake with mature as compared to heading stage hay are unknown at this time but may be related to differences in alkaloid levels in the fescue hays.

Table 1. Chemical composition of tall fescue hays and supplements.

Item	Vegetative	Boot Stage	Heading Stage	Mature	Soybean Hulls	Corn
Organic matter, % DM	90.7	92.3	92.5	93.7	93.8	98.3
Crude protein, % DM	17.4	15.6	8.2	7.8	13.9	9.0
Neutral detergent fiber, % DM	68.7	73.9	73.8	76.8	64.3	12.0
Acid detergent fiber, % DM	34.4	37.9	42.3	43.2	46.2	3.4
Lignin, % DM	5.2	5.2	5.6	5.3	2.2	0.7

Supplementation decreased (P < 0.01) forage DMI and increased (P < 0.01) total DMI, whereas no differences between supplements were detected (P > 0.10) for either response variable. Digestible DMI was greater (P < 0.01) with than without supplementation and greater (P < 0.01) with soybean hulls than with corn.

The supplement type x hay maturity interaction was significant (P = 0.08) for apparent total-tract DM digestibility (DMD; Figure 4). Supplementing vegetative hay had no effect (P > 0.10) on DMD. For boot stage hay, supplementation increased (P < 0.01) DMD compared to control, and no differences (P > 0.10) were detected between the two supplement types. With the heading stage and mature hays, supplementation also increased (P ≤ 0.01) DMD, and the increase was greater for soybean hulls than for corn. A supplement type x hay maturity interaction was also observed (P < 0.05) for NDF digestibility (Figure 5). As with DMD, no effect of supplementation was observed for the earliest forage maturity. With boot stage hay, corn supplementation depressed (P < 0.05) NDF digestibility. With heading stage hay, soybean hull supplementation resulted in significantly higher (P < 0.01) NDF digestibility than corn or no supplement, and corn supplementation depressed (P < 0.05) NDF digestion below that of hay alone. For mature hay, no difference (P > 0.10) was detected between corn and no supplementation, and soybean hull supplementation increased (P < 0.02) NDF digestion.

Substitution effects of energy supplementation were measured by determining the unit change in forage intake per unit of supplement offered. Negative values indicate a depression in forage intake with supplement consumption. For all hay maturities, substitution ratios did not differ (P > 0.10) for soybean hulls (average = -0.45) and corn (average = -0.50).

Nitrogen retention was calculated as the difference between N intake and N excretion in the urine and feces. A supplement x hay interaction (P < 0.10) was observed for N retention. For vegetative hay, no supplementation effects were found (P > 0.10). For all other hays, supplementation increased N retention (P < 0.10) relative to control, with the exception that corn supplementation only tended (P = 0.13) to increase N retention with mature hay. No differences (P > 0.10) were seen between corn and soybean hulls relative to their effects on N retention.

Forage maturity effects on N retention were similar to effects on intake, decreasing (P < 0.10) with advancing maturity, with the exception that no differences were noted between heading stage and mature hays. Increases in N retention with corn or soybean hull supplementation were likely related to increased capture of ruminal N and incorporation into microbial protein. Thus, it appears that the vegetative hay had sufficient digestible energy to support microbial growth, as no increases in N retention were observed when this hay was supplemented.

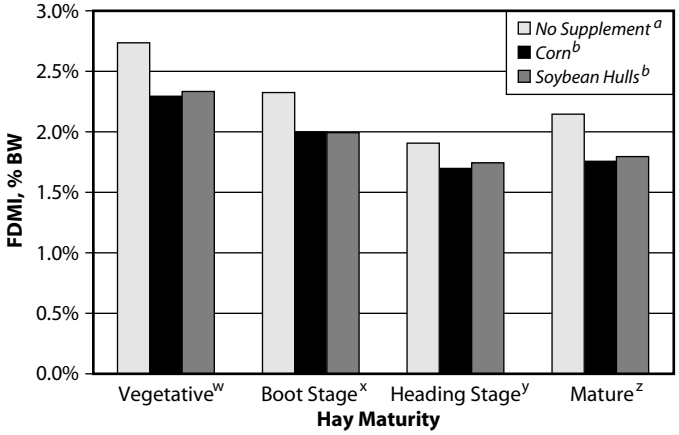
Ruminal Kinetics and Fermentation

No supplement type x hay maturity interaction was found (P > 0.10) for liquid dilution rate. Supplementation tended (P = 0.19) to increase dilution rate, whereas hay maturity had a significant effect. This effect matched closely with effects on intake in that dilution rate decreased (P < 0.05) with increasing maturity for vegetative through heading stage hays, yet increased slightly when maturity advanced from heading stage to mature. Although reasons for this increase are unknown, the data do provide support for the intake effects noted earlier. Greater passage rates are typically associated with greater intake.

Supplement type x sampling time interactions (P < 0.10) were found for all ruminal fermentation data. However, these interactions existed because of differences in the magnitude of response at various sampling times rather than as a consequence of different treatment rankings. Thus, we have presented the data averaged across sampling times.

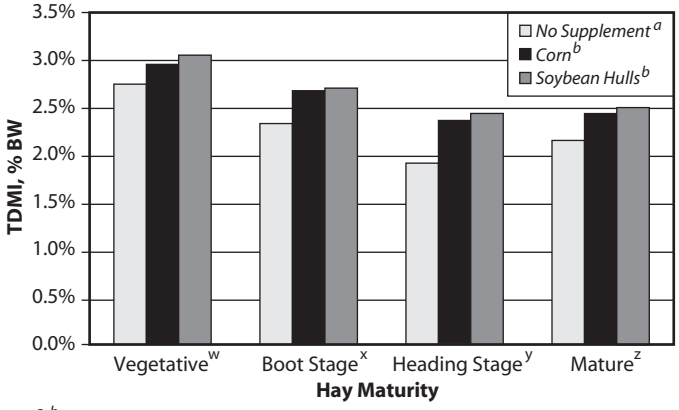
There were no supplement type x hay maturity interactions (P > 0.10) for any of the fermentation variables presented. Ruminal pH (Tables 2 and 3) was unaffected (P > 0.10) by supplementation and increased (P < 0.10) with advancing forage maturity, with the exception that no differences were seen between

Figure 1. Effect of hay maturity and supplement type on forage dry matter intake (FDMI) as a percent of body weight (BW). Supplement type x hay maturity interaction (P = 0.43).



a, b Supplement type means with different superscripts differ (P < .01).
w, x, y, z Hay maturity means with different superscripts differ (P < .01).

Figure 2. Effect of hay maturity and supplement type on total dry matter intake (TDMI) as a percent of body weight (BW). Supplement type x hay maturity interaction (P = 0.43).



a, b Supplement type means with different superscripts differ (P < .05).
w, x, y, z Hay maturity means with different superscripts differ (P < .02).

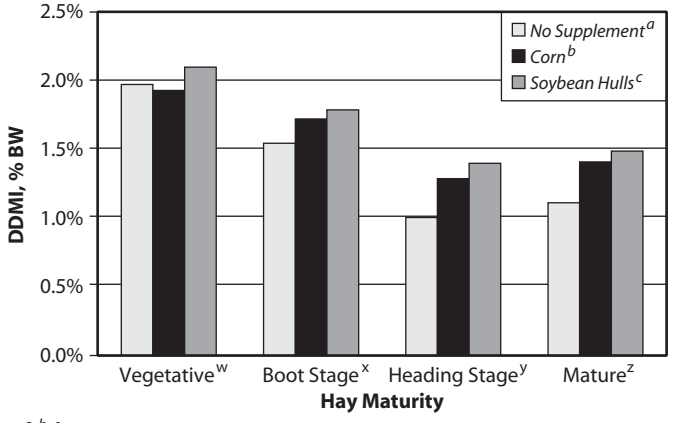
heading stage and mature hays. Even with no supplementation, ruminal pH for the vegetative hay averaged below 6.2, a level sometimes considered to be low enough to inhibit fiber digestion. Although pH tended to be slightly lower (numerically) with corn than with soybean hull supplementation, with most hays the magnitude of the difference was small, making it unlikely that ruminal pH was an important mediator of depressed fiber digestion with corn supplementation.

Ruminal NH_3 concentrations also were affected ($P < 0.01$) by hay maturity. However, this effect was unique among the response variables we measured in that NH_3 concentrations were greatest ($P < 0.01$) with boot stage hay. Ruminal NH_3 concentrations are a function of NH_3 release into the rumen (from both dietary N and recycled urea) and NH_3 uptake, primarily via incorporation into microbial cells and absorption from the rumen. Thus, the increase in NH_3 concentration from vegetative to boot stage hay may represent lower ruminally available energy:nitrogen ratios with the boot stage hay. A decrease in ruminally available energy would limit microbial growth and subsequent uptake of NH_3 by the ruminal microorganisms.

Ruminal NH_3 concentrations were lower ($P < 0.10$) with corn supplementation than with soybean hulls or no supplement. Although slightly greater protein levels were supplied with soybean hulls than with corn, we believe much of this effect is a consequence of energy substrate. Other work from our laboratory has shown that semi-purified corn starch administered to the rumen has a greater effect on lowering ruminal NH_3 concentrations than does semi-purified, delignified, digestible fiber.

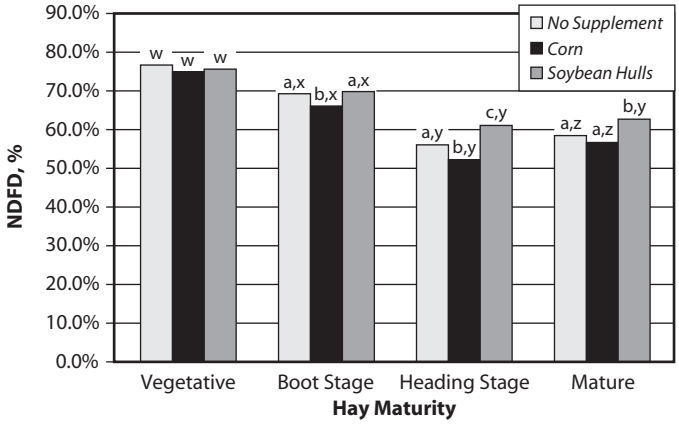
Ruminal VFA concentrations were rather high (corresponding with low pH values) on all treatments and were greater ($P < 0.01$) for vegetative and boot stage hays than for the two more mature hays. Supplementation did not affect ($P > 0.10$) VFA

Figure 3. Effect of hay maturity and supplement type on digestible dry matter intake (DDMI) as a percent of body weight (BW). Supplement type x hay maturity interaction (P = 0.54).



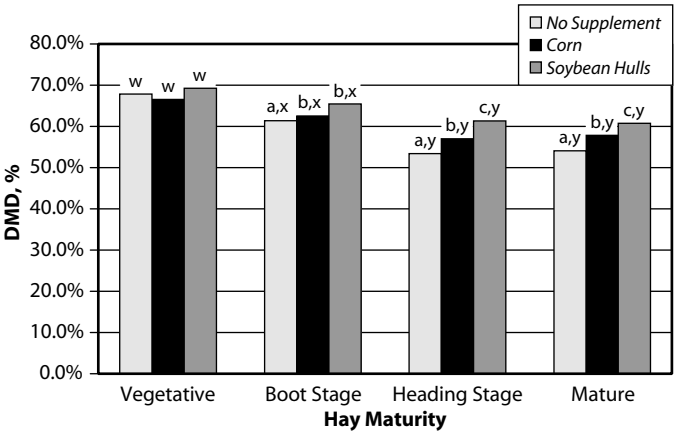
a, b, c Supplement type means with different superscripts differ (P < .03)
w, x, y, z Hay maturity means with different superscripts differ (P < .01).

Figure 5. Effect of hay maturity and supplement type on neutral detergent fiber digestibility (NDFD) as a percent. Supplement type x hay maturity interaction (P = 0.04).



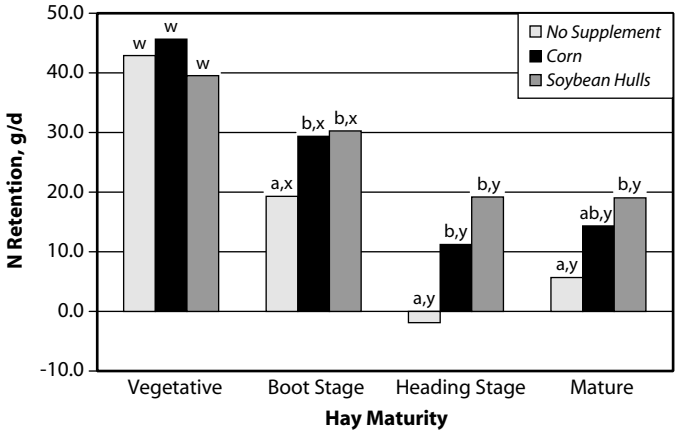
a, b, c Means within a hay maturity with different superscripts differ (P < .05).
w, x, y, z Means within a supplement type with different superscripts differ (P < .09).

Figure 4. Effect of hay maturity and supplement type on dry matter digestibility (DMD) as a percent. Supplement type x hay maturity interaction (P = 0.08).



a,b, c Means within a hay maturity with different superscripts differ (P < .08).
w, x, y, z Means within a supplement type with different superscripts differ (P < .01).

Figure 6. Effect of hay maturity and supplement type on nitrogen retention in g retained N per day. Supplement type x hay maturity interaction (P = 0.10).



a, b, c Means within a hay maturity with different superscripts differ (P < .08).
w, x, y, z Means within a supplement type with different superscripts differ (P < .06).

concentrations, although they were numerically greater with soybean hulls than with the other treatments. Molar proportions of acetate responded similarly to VFA concentrations with advancing forage maturity, although the magnitude of the differences was small, averaging 74.4% for the two less mature hays and 73.0% for the two more mature hays.

Relative to control, both supplementation treatments depressed ($P < 0.05$) acetate proportions, with a greater ($P < 0.05$) depression with corn than with soybean hulls. Again, however, the magnitude of these effects was small. No significant effects of supplementation were detected ($P > 0.10$) for ruminal propionate proportions, and these proportions were greater ($P < 0.10$) for heading stage hay than for the earliest two maturities of hay. Like acetate proportions, the magnitude of the differences did not exceed 1 percentage unit. Acetate:propionate ratios responded in kind, with no effect ($P > 0.10$) of supplementation, and slightly lower values ($P < 0.10$) for the two more mature hays.

Table 2. Influence of hay maturity on ruminal kinetic and fermentation measures.

	Hay Maturity				
Item	Vegetative	Boot Stage	Heading Stage	Mature	SEM[a]
Liquid dilution rate, %/h	10.4[c]	8.9[d]	8.0[e]	8.7[d]	0.43
Ruminal pH	6.16[c]	6.28[d]	6.45[e]	6.42[e]	0.03
Ruminal NH_3, mM	7.3[c]	13.5[d]	6.7[c]	4.9[e]	0.66
Ruminal VFA, mM	123.0[c]	121.3[c]	105.9[d]	105.1[d]	2.19
A:P[b]	4.5[c]	4.5[c]	4.2[d]	4.3[d]	0.08
	moles/100 moles				
Acetate	73.2[c]	73.4[c]	71.9[d]	72.4[d]	0.30
Propionate	16.5[c]	16.4[c]	17.2[d]	17.0[cd]	0.28

a SEM = standard error of the mean (n = 12).

b A:P = acetate:propionate ratio.

c, d, e, f Means within a response variable with different superscripts differ ($P < 0.10$).

Table 3. Influence of supplement type on ruminal kinetic and fermentation measures.[a]

	Supplement Type			
Item	No Supplement	Corn	Soybean Hulls	SEM[b]
Liquid dilution rate, %/h	8.1	9.1	9.8	0.62
Ruminal pH	6.33	6.28	6.37	0.04
Ruminal NH_3, mM	8.3[d]	6.1[e]	9.9[d]	0.78
Ruminal VFA, mM	113.7	109.7	118.0	3.39
A:P[c]	4.4	4.3	4.4	0.08
	moles/100 moles			
Acetate	73.7[d]	71.7[e]	72.8[f]	0.28
Propionate	16.7	17.0	16.6	0.28

a Sampling time x supplement type interactions existed ($P \leq 0.10$) for ruminal pH, NH_3, VFA, Acetate:Propionate, and molar proportions of acetate and propionate. These interaction means are not shown here (refer to text for explanation).

b SEM = standard error of the mean (n = 16).

c A:P = acetate:propionate ratio.

d, e, f Means within a response variable with different superscripts differ ($P < 0.10$).

Conclusion

Interactions that were detected between forage maturity and supplement type for digestibility and N retention support the concept that relative advantages of fiber supplementation are greater with lower-quality forages. However, no such interactions were noted for the key response variables of total and digestible DMI. These responses suggest that energy supplementation will increase growing cattle performance regardless of forage quality, and that when supplemented near the levels used in this study, a given amount of pelleted soybean hulls will support a greater level of performance than an equal quantity of cracked corn grain.

Summary

A metabolism study was conducted using ruminally cannulated steers to study whether maturity of tall fescue hay affected intake and utilization responses to corn or soybean hull supplements. Supplement type x hay maturity interactions were not detected ($P > 0.10$) for hay, total, or digestible DMI, which generally decreased ($P < 0.01$) as maturity increased. Supplementation decreased ($P < 0.01$) hay DMI and increased ($P < 0.01$) total and digestible DMI. Digestible DMI was greater ($P = 0.03$) with soybean hulls (1.7% BW) than with corn (1.6% BW), both of which were greater ($P = 0.02$) than with no supplement (1.4% BW). Supplement x maturity interactions ($P < 0.10$) were observed for DM and NDF digestibilities. Differences in digestibilities between corn and soybean hulls increased with advancing forage maturity.

For ruminal pH, total VFA concentrations, and molar proportions of most VFA, the supplement x forage maturity x sampling time interaction was not significant ($P > 0.10$), whereas the supplement x time and maturity effects were significant ($P < 0.01$). Only minor differences ($P < 0.10$) in ruminal pH due to supplement type were detected at any sampling time, and pH increased with advancing forage maturity (6.16, 6.28, 6.45, and 6.42 for vegetative, boot stage, heading, and mature hays, respectively).

Grazing Corn versus Commodity Feeding for Backgrounding Feeder Cattle

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Introduction

Backgrounding or growing of feeder cattle from weaning to yearling weights provides Kentucky producers with an alternative beef enterprise. Cattle can be purchased in the fall, wintered, and sold in spring, allowing producers to take advantage of historically positive changes in market prices. Winter forage that can provide high levels of performance through grazing is not available in Kentucky due to climatic conditions; thus, stored and/or purchased feeds must be used, resulting in increased cost and labor requirements.

Corn chopped and stored as a silage crop maximizes nutrient production and the number of cattle that may be backgrounded per acre. To produce corn silage requires a large investment in equipment and storage structure and is generally not feasible for medium to small-size producers. Feeding of commodities or grain by-products has gained popularity in recent years due to low costs and ready availability. Producers with limited land resources have been able to use commodities and increase the number of cattle backgrounded.

Commodity prices have risen sharply during the last year, resulting in increased costs of production for smaller producers who cannot purchase in truckload lots. Due to rising prices, smaller producers are looking for alternative feeding systems that will allow continued, economical backgrounding of cattle.

Recent field demonstrations of grazing standing corn during fall and early winter have shown that cattle will perform well with reasonable feed utilization. This field study was conducted to determine performance and economics of steers grazing standing corn and to compare with like steers fed a commodity and hay ration in pasture drylot.

Procedures

One hundred and fifty-six steers were purchased from area sale barns for this trial. All steers were treated with standard health procedures for the BRD complex, dewormed with Cydectin (Fort Dodge Animal Health), and implanted with Synovex S (Fort Dodge Animal Health). All calves were retained in one group and preconditioned until trial initiation on September 27, 2000.

Steers were individually identified by ear tag and randomly allocated by weight to either the corn grazing or commodity feed treatment. Individual weights were taken at trial initiation, Days 32 and 65, and at trial termination on Day 78. Due to pretrial marketing requirements, the heaviest steers were removed and sold at Weigh Days 32 and 65. All remaining steers were sold at trial termination on Day 78.

Corn acreage was measured by GPS and totaled 11.8 acres. Grain yield averaged 172.8 bushels per acre, and total biomass yielded 9.25 tons of dry matter per acre. The field was strip-grazed in five sections utilizing temporary electric fencing and moving away from the water source with each new section. This allowed cattle continued access to previously grazed sections. All corn plant material within a 6.25-square-foot quadrant from four randomly selected sites was picked up, separated, and weighed to determine utilization each time the cattle were allowed access to a new site.

The difference between grain and biomass yield prior to grazing and post-grazing grain and biomass yield was considered as utilization. Water was provided in insulated waterers connected to countywide rural water. Protein tubs were provided at the water site to promote consumption.

Commodity treatment cattle were provided with free-choice access to round rolls of hay and fed a blend consisting of equal parts soybean hulls and corn gluten feed. Commodities were fed ad libitum once daily in troughs. Bunk space per head was adequate and did not limit feed intake or performance of cattle.

Cattle prices used in the economic analysis were taken from data reported in the *Kentucky Livestock and Grain Report,* Kentucky Department of Agriculture, for the respective dates.

Results and Discussion

Acres per section, sampling date, and percent of utilization of grain and total biomass (grain, ear, stalk, and leaves) are shown in Table 1. Observation and percent of utilization during Period 1 indicate that cattle did not immediately begin full consumption of the corn. Cattle appeared not to understand how to eat the plant and continually walked the boundary. Decreased biomass utilization during Periods 3 and 4 indicate a deliberate attempt by the researchers to decrease consumption of less desirable parts of the corn plant and improve performance of the cattle. A weighted average utilization of grain was 78.3%. Figures in Table 1 more accurately represent disappearance rather than utilization, as some material was undoubtedly walked into the ground, but for this paper, it will be considered as utilization.

Gain of cattle on trial for 32, 65, and 78 days is shown in Table 2. No significant difference in performance due to treatment was detected in this trial. Cattle in both the corn grazing and commodity treatment group lost weight during the first 32 days of the trial. As stated earlier, cattle grazing standing corn did not consume the crop readily. Some sickness was encountered with both groups even though they had been preconditioned, possibly resulting in lowered initial performance. Gain of cattle on trial for either 65 days or the entire period was acceptable. This increase in gain may reflect improved health; adaptation to grazing corn; removal of dominant but poorly

Table 1. Percent utilization of grain and total biomass by steers grazing standing corn.

Period	Acres	Sampling Date	Percent Utilization	
			Grain	Biomass
1	1.8	10/9	55.2	36.1
2	2.5	10/27	84.9	79.8
3	1.9	11/17	81.9	53.5
4	3.1	12/7	81.0	44.0
5	2.5	—	—	—

performing cattle, allowing lighter-weight, faster-gaining cattle to be expressed; or additional factors not well understood.

Estimated average daily feed intake/disappearance is shown in Table 3. Values were determined by direct weights of the blended commodity feed, counting hay rolls provided with an average estimated weight and counting bags of mineral and number of protein tubs provided. Corn grain consumption was estimated by yield and a weighted utilization of 78.3%. It should again be emphasized that these values, particularly corn grain and hay, are estimates of disappearance, not necessarily consumption. These values are important for financial analysis whether consumed or not because they were presented to the groups and must be accounted for economically.

Prices of feeds are shown in Table 4, and cattle prices and values used in economic comparisons are shown in Table 5. Corn price was the actual out-of-pocket expense for the 11.8 acres planted. Purchase price was used for other ingredients.

An economic comparison of backgrounding cattle with corn grazing or commodity feeding is shown in Table 6. Cattle grazing corn had a positive net return of $21.04 per head. Feed cost per pound of gain was $0.34 for these cattle. When economic calculations were made on the market value of corn if it had been harvested and sold as grain (Table 7), the cattle lost a small amount of money. Conversion of corn to gain was excessively high. Many smaller producers do not have the equipment or facilities to harvest and store corn grain, and a cash market does not exist in many small rural communities, so the positive net return over cash expenses is meaningful for them. Cattle consuming the commodity blend lost $10.45 per head on a feed cost per pound of gain of $0.645 cents. The commodity cattle did not gain well, particularly early in the trial. Previous field trials at this level of commodity consumption have yielded gains well in excess of two pounds daily.

Summary

Steers grazing standing corn performed similarly to steers fed a blended commodity ration with rolls of hay. Grazing standing corn was profitable when returns were expressed against variable costs. Grazing standing corn may be an alternative for smaller producers without silage equipment or storage to background increased numbers of cattle if commodity prices remain at high levels. Additional research with cattle performance at higher levels is necessary before conclusions are reached and the practice widely adopted.

Table 2. Performance of cattle grazing corn or fed commodities and hay.[a]

	Treatment					
	Corn Grazing			Commodity Feeding		
Days	32	65	78	32	65	78
Head[b]	82	50	46	74	46	43
Initial wt, lb	687	669	639	690	672	639
Final wt, lb	667	775	785	687	757	787
ADG, lb	-0.62	1.62	1.88	-0.08	1.27	1.87

[a] Least square means.

[b] All animals remaining at weight period, not only those marketed.

Table 3. Average daily feed consumption/disappearance.

Treatment	Corn Grazing	Commodity Feeding
Corn grain, lb	18.4	—
Commodity blend, lb	—	12.9
Hay, lb	—	12.8
Protein tub, lb	1.52	—
Mineral, oz	2.13	4.0

Table 4. Feed prices used for economic analysis.

Feed	Price
Hay	$20/roll
Protein tub	$24/tub
Commodity blend	$100/ton
Mineral	$12.45/bag
Corn	$125/acre

Table 5. Cattle prices and income.

	Treatment					
	Corn Grazing			Commodity Feeding		
Trial days	32	65	78	32	65	78
Number sold	32	4	46	28	3	43
Purchase price, $	84.55	86.95	87.27	84.13	86.23	87.27
Sale price, $	87.86	82.90	83.25	86.59	84.94	83.17
Gross/head, $	30.95	56.20	96.68	34.34	35.90	96.88
Gross/group, $	990.40	224.80	4,447.28	961.52	107.70	4,165.84
Gross/trt, $	—	5,662.48	—	—	5,235.06	—
Average gross/head, $	—	69.05	—	—	70.74	—

Table 6. Economics of corn grazing and commodity feeding for backgrounding cattle.

Treatment	Corn Grazing	Commodity Feeding
Gross income/head, $	69.05	70.74
Expense/head, $		
Health	8.25	8.25
Feed	30.79	61.53
Labor[a]	1.87	4.25
Interest (calf)	7.10	7.16
Net return/head, $	21.04	(10.45)

[a] Labor amounts of 45 hours for commodity feeding and 22 hours for corn grazing at $7/hour.

Table 7. Economics of corn grazing with corn grain priced at market value.[a]

Treatment	Corn Grazing
Gross income/head, $	69.05
Expense/head, $	
Health	8.25
Feed	52.59
Labor	1.87
Interest (calf)	7.10
Net return/head, $	(0.76)

[a] Corn grain valued at $1.60/bu from *Kentucky Livestock and Grain Report*, Vol. 14, Issue 38, 9/22/00.

Starch and Digestible Fiber as Supplements for Cattle Grazing Fescue

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Introduction

One approach to increasing performance of grazing cattle is to provide supplemental feed. Generally, with cool-season pastures like tall fescue, protein is not limiting; therefore, energy supplements are the best means to boost gains, and grain supplements are the most common type of energy supplement. However, starch is the main energy source in feed grains and for decades nutritionists have recognized that adding starch to forage-based diets results in decreased intake and digestion of the forage.

In recent years, there has been considerable interest in using by-products that have large amounts of highly digestible fiber as energy supplements. By-products often represent an inexpensive source of energy, and, with fiber-based supplements, we can get around some of the negative effects that we see with starch-based supplements.

Several research studies have compared starch- and fiber-based supplements with varying results. Part of the variation comes from the fact that the effects of supplementation appear to depend on the composition of the forage consumed. Without information on specific forage types, it is difficult to predict responses in forage intake and use.

These experiments were conducted to determine how digestible fiber- and starch-based supplements affect intake and utilization of grazed, endophyte-infected, tall fescue in the fall (high-quality forage) and summer (low-quality forage). In order to remove potential effects due to other supplemental constituents (for example, different amounts and sources of protein) and to study responses due to energy source per se, we conducted these experiments with semi-purified supplements.

Procedures

We used 12 ruminally cannulated Angus steers in each of two experiments. In the first experiment, all steers grazed a 3.04-ha (7.5-ac), stockpiled, Kentucky 31 tall fescue (*Festuca arundinacea*) pasture in December. In the second experiment, conducted during June, steers grazed mature, Kentucky 31 tall fescue in a separate 3.04-ha pasture. Endophyte infestation levels averaged 67% and 77% in Experiments 1 and 2, respectively. In both experiments, pastures were fertilized with ammonium nitrate at 56 kg N/ha (50 lb N/ac) in both March and June.

Steers in both experiments had free-choice access to fresh water and a mineral/vitamin supplement (18% salt, 13.0% Ca, 6.2% P, 3.0% Mg, 1.0% S, 0.8% K, 2300 ppm Zn, 175 ppm Fe, 2200 ppm Mn, 1070 ppm Cu, 55 ppm I, 11 ppm Co, 29 ppm Se, 661,390 IU/kg vitamin A, and 276 IU/kg vitamin E).

Both experiments were conducted similarly. Body weights were obtained at the beginning and end of each experiment, following an overnight stand without access to feed or water. In both experiments, steers were randomly assigned, within weight blocks, to the following treatments:

- Control—no supplementation;
- Starch—corn starch (Cargill, Minneapolis, MN) ruminally administered at 0.75% of BW daily; and
- Fiber—delignified oat fiber ruminally administered at 0.75% of BW daily.

Steers were gathered from pasture daily at approximately 0900 and ruminally dosed with their respective supplements. Adaptation (Experiment 1, Days 1 through 11; Experiment 2, Days 1 through 14) was followed by four days of ruminal evacuations and masticate sampling, then six days of total fecal collection using fecal bags, and one day of ruminal fluid sampling for characterization of ruminal pH, ammonia N, volatile fatty acid (VFA) concentrations, and liquid dilution rate, using dosed chromium EDTA as a liquid marker.

Fecal and ruminal digesta samples and samples of supplements were dried to a constant weight in a forced-air oven at 55°C. Masticate samples were freeze dried. All of these samples were ground through a 1-mm screen with a Wiley mill and analyzed for dry matter (DM) and organic matter (OM). Crude protein (CP) concentrations (using gas N analysis) and protein degradability (using an enzymatic procedure with a *Streptomyces griseus* protease) were determined for the masticate samples. Concentrations of ash-free neutral detergent fiber

(NDF) and acid detergent fiber (ADF) in supplement, masticate, and fecal samples were measured using an ANKOM fiber analyzer. Indigestible ADF (IADF) was measured in supplement, masticate, fecal, and ruminal digesta samples as an indigestible marker for calculation of digestibilities, intake, and passage rate.

Data were analyzed using a model for a randomized complete block design with the MIXED procedure of SAS. Treatment was included as a fixed effect and block as a random effect. Means were separated using a protected ($P < 0.10$) Fisher's LSD.

Repeated measures (including ruminal fermentation characteristics and IADF passage rates) were analyzed using the MIXED procedure of SAS, with treatment and sampling time specified as fixed effects and block and the treatment x block interaction as random effects. Orthogonal polynomial contrasts were used to identify linear, quadratic, and cubic time effects. When significant time x treatment interactions existed, contrasts were used to identify significant treatment effects within sampling times and time effects within individual treatments.

Results and Discussion

It is uncommon for dietary supplementation to have substantial effects on the quality of diet selected by grazing cattle. Accordingly, we saw no effects of treatments on the chemical composition of masticate samples collected by steers grazing stockpiled fescue (Table 1) and only minor effects for cattle grazing summer fescue (Table 2). In this case, fiber concentrations were slightly greater (about 3 percentage units) for cattle receiving the starch supplement than for the cattle receiving fiber supplement. Reasons for greater dietary fiber concentrations with starch supplementation in this experiment are not known. However, it is unlikely that this small shift in diet composition would have substantial effects on animal performance.

Many factors affect the chemical composition of grazed forage, including forage maturity and pasture fertilization. In these experiments, CP concentration averaged 18.2% of OM for stockpiled fescue and 11.2% of OM for summer fescue. These values, as well as the NDF and ADF values, were similar to other reported values, particularly after considering that masticate samples (samples actually collected by the grazing animals) will have higher CP and lower fiber values than clipped or hay samples from the same pastures.

Forage intake (Tables 3 and 4) was decreased with starch, but not with fiber supplementation in both experiments. Intake estimates in the first experiment were lower than expected, and this is likely related to the marker techniques used to estimate intake. Despite this, relative differences between the treatments within each experiment should be valid. Substitution ratios, which indicate the change in forage intake per unit change in supplement intake, indicate that adding digestible fiber had very little influence on voluntary forage intake, whereas starch supplementation at 0.75% of body weight resulted in a 1-to-1 substitution for forage intake. Thus, total and digestible OM intakes were increased (nonsignificantly in Experiment 2) when fiber was supplemented but were unaffected with starch supplementation.

Table 1. Composition of diets selected by steers grazing stockpiled fescue and supplemented with starch or fiber (Experiment 1).[a]

	Treatment				
Item	Control	Fiber	Starch	SEM[b]	P Value[c]
OM, % of DM	87.4	86.1	87.4	0.60	0.23
CP, % of OM	18.3	18.0	18.4	0.60	0.88
DIP, % of CP	83.9	84.2	82.4	0.98	0.42
NDF, % of OM	61.2	65.8	59.1	2.74	0.14
ADF, % of OM	27.9	29.8	27.8	0.96	0.20

a DM = dry matter; OM = organic matter; CP = crude protein; DIP = degradable intake protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.
b Standard error of the mean (n = 4).
c Probability of a greater F-value for overall treatment F-test.

Table 2. Composition of diets selected by steers grazing summer fescue and supplemented with starch or fiber (Experiment 2).[a]

	Treatment				
Item	Control	Fiber	Starch	SEM[b]	P Value[c]
OM, % of DM	86.8[d]	87.3[d,e]	88.2[e]	0.60	0.09
CP, % of OM	11.3	11.2	11.2	0.43	0.98
DIP, % of CP	73.0	75.0	74.4	1.12	0.50
NDF, % of OM	67.2[d,e]	66.3[d]	69.1[e]	0.74	0.06
ADF, % of OM	33.4[d]	32.8[d]	35.0[e]	0.76	0.06

a DM = dry matter; OM = organic matter; CP = crude protein; DIP = degradable intake protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.
b Standard error of the mean (n = 4).
c Probability of a greater F-value for overall treatment F-test.
d,e Means within a row with different superscripts differ ($P < 0.10$).

Effects on digestible OM intake indicate that fiber, but not starch supplementation, would be expected to promote greater energy intake and animal performance. With stockpiled fescue, forage and NDF digestibilities were decreased with both supplements, although mechanisms for this response appeared to be different for fiber than for starch. With fiber supplementation, passage rate (as measured with IADF) was increased, which would be expected to result in decreased digestibility. With starch supplementation, this was not the case, and liquid passage actually decreased.

Many mechanisms have been proposed for decreased fiber digestion with starch supplementation, including decreased ruminal pH and decreased availability of microbial nutrients such as NH_3. Ruminal pH (Table 5) was lower with fiber than with starch supplementation, yet NDF digestion was lowest with starch. This indicates that some factor other than pH depression was involved with the depression in fiber digestion. Ruminal NH_3 concentrations were lowest with starch, suggesting that limited NH_3 may have contributed to the depression in fiber digestion.

With the lower-quality forage in Experiment 2, fiber supplementation did not affect passage rate, forage OM digestibility, or NDF digestibility. However, starch supplementation again depressed forage and NDF digestibilities, concurrent with decreased ruminal NH_3 concentrations (Table 6). In this case, ru-

minal pH was also decreased with starch but not with fiber supplementation. Thus, in this experiment, we cannot exclude ruminal pH as a primary mechanism in the depression of fiber digestion.

Ruminal fermentation characteristics are shown in Tables 5 and 6. The greatest ruminal VFA concentrations occurred with fiber supplementation on stockpiled fescue and with starch supplementation on summer fescue, mirroring changes in ruminal pH. In both experiments, molar proportion of acetate was decreased, and molar proportion of butyrate was increased with starch supplementation. Very little change was measured in propionate proportions in either study, and acetate:propionate ratios were unaffected by treatment.

Although fiber effects were fairly consistent between experiments, effects of starch on proportions of the minor VFA, valerate, isobutyrate, and isovalerate differed between the two experiments. With stockpiled fescue, there was a dramatic increase in valerate and a lesser, though significant, increase in isobutyrate proportion with starch. Reasons for this shift are unclear, although it is possible that, with these particular dietary conditions, growth of valerate- and isobutyrate-producing microorganisms was facilitated. With summer fescue, we detected no affect of starch supplementation on valerate and small decreases in isobutyrate and isovalerate proportions.

Very little research has been done evaluating degradability of protein in fescue. Values in these experiments (83.5% and 74.1% of the CP with stockpiled and mature fescue, respectively) were somewhat greater than expected, based on other work with cool-season grasses. Generally, the moderate to high CP concentrations coupled with the large protein degradability values suggest that excess degradable protein exists in these forages (particularly in the stockpiled fescue) relative to available energy. One means of capturing excess degradable protein is to supply additional ruminally available energy with supplements. The decreases in ruminal ammonia concentrations (Tables 5 and 6) with supplementation in both experiments suggest that a greater proportion of the degradable protein was captured in the form of microbial protein when supplements were fed. Thus, we would expect less wastage of feed protein when energy supplements are fed, and less with starch than with fiber supplementation.

Table 3. Influence of supplementation with starch or fiber to steers grazing stockpiled fescue on intake and digestibility (Experiment 1).[a]

	Treatment				
Item	**Control**	**Fiber**	**Starch**	**SEM[b]**	**P Value[c]**
Initial body weight, kg	389.0	381.0	393.5	19.91	0.51
Final body weight, kg	385.0	397.5	394.0	18.26	0.56
Forage OMI, % BW	1.55[d]	1.58[d]	0.79[e]	0.051	< 0.01
Supplement OMI, % BW	0.0	0.71	0.75	—	—
Total OMI, % BW	1.55[d]	2.29[e]	1.54[d]	0.049	< 0.01
Substitution ratio	—	0.04[d]	-1.01[e]	0.077	< 0.01
Digestible OMI, % BW	1.11[d]	1.51[e]	1.17[d]	0.049	< 0.01
Forage OMD, %	71.6[d]	61.8[e]	56.5[e]	3.66	0.05
Total OMD, %	71.6[d]	65.9[e]	75.8[f]	1.52	< 0.01
NDFD, %	75.1[d]	68.1[e]	54.8[f]	2.11	< 0.01
Ruminal DM fill, % BW	1.07	1.11	1.01	0.074	0.60
Ruminal liquid fill, mL/kg BW	97.0	97.6	87.3	6.11	0.40
IADF passage rate, %/h	3.25[d]	4.48[e]	2.30[d]	0.468	0.03
Liquid dilution rate, %/h	14.6[d]	14.6[d]	8.7[e]	1.37	< 0.01

a OMI = organic matter intake; BW = body weight; Substitution ratio is unit change in forage OMI per unit change in supplement OMI. Positive values represent an increase in forage intake, negative values represent a decrease in forage intake with supplementation; OMD = organic matter digestibility; NDFD = neutral detergent fiber digestibility; DM = dry matter; IADF = indigestible ADF.

b Standard error of the mean (n = 4).

c Probability of a greater F-value for overall treatment F-test.

d, e, f Means within a row with different superscripts differ (P < 0.05).

Table 4. Influence of supplementation with starch or fiber to steers grazing summer fescue on intake and digestibility (Experiment 2).[a]

	Treatment				
Item	**Control**	**Fiber**	**Starch**	**SEM[b]**	**P Value[c]**
Initial body weight, kg	243.8	241.0	248.9	24.91	0.73
Final body weight, kg	250.5	245.5	269.3	25.09	0.55
Forage OMI, % BW	2.32[d]	2.13[d,e]	1.65[e]	0.202	0.10
Supplement OMI, % BW	0.0	0.69	0.68	—	—
Total OMI, % BW	2.32	2.82	2.32	0.203	0.19
Substitution ratio	—	-0.28[d]	-1.00[e]	0.248	0.09
Digestible OMI, % BW	1.51	1.91	1.54	0.167	0.23
Forage OMD, %	64.6[d]	65.1[d]	52.9[e]	2.78	0.03
Total OMD, %	64.6	67.6	65.7	1.65	0.43
NDFD, %	66.7[d]	69.2[d]	54.2[e]	2.31	< 0.01
Ruminal DM fill, % BW	2.43	2.23	2.22	0.146	0.39
Ruminal liquid fill, mL/kg BW	170.5	161.6	149.4	8.16	0.24
IADF passage rate, %/h	2.55	2.60	2.35	0.275	0.75
Liquid dilution rate, %/h	10.6	12.2	12.6	1.06	0.39

a OMI = organic matter intake; BW = body weight; Substitution ratio is unit change in forage OMI per unit change in supplement OMI. Positive values represent an increase in forage intake, negative values represent a decrease in forage intake with supplementation; OMD = organic matter digestibility; NDFD = neutral detergent fiber digestibility; DM = dry matter; IADF = indigestible ADF.

b Standard error of the mean (n = 4).

c Probability of a greater F-value for overall treatment F-test.

d, e, f Means within a row with different superscripts differ (P < 0.05).

Summary

Two experiments were conducted with grazing, ruminally cannulated steers to study the influence of starch or digestible fiber supplements on grazed forage intake and use. Regardless of whether steers were grazing stockpiled fescue in the winter or mature fescue in the summer, starch supplementation caused a dramatic depression in forage intake, whereas forage intake was unaffected when digestible fiber was supplemented. Also, starch supplementation resulted in the greatest depression in fiber digestion in both experiments. However, effects of starch and fiber supplementation on digestibility, passage rates, and ruminal fermentation characteristics differed with differences in forage quality. Results indicate that fiber-based supplementation offers advantages over starch-based supplementation for grazing cattle.

Table 5. Influence of supplementation with starch or fiber to steers grazing stockpiled fescue on ruminal fermentation characteristics (Experiment 1).[a]

	Treatment				
Item	**Control**	**Fiber**	**Starch**	**SEM[b]**	**P Value[c]**
VFA concentration, mM[d]	78.6[e]	94.5[f]	71.8[e]	4.64	< 0.01
Acetate:Propionate[d]	4.78	4.53	4.53	0.253	0.70
NH_3, mM[d]	7.6[e]	3.0[f]	0.6[g]	0.36	< 0.01
pH[d]	6.57[e]	6.26[f]	6.47[g]	0.042	< 0.01
	moles/100 moles				
Acetate[d]	75.2[e]	75.4[e]	67.9[f]	0.66	< 0.01
Propionate[d]	15.8	16.7	16.0	0.69	0.61
Butyrate[d]	6.9[e]	6.6[e]	10.0[f]	0.57	< 0.01
Valerate[d]	0.59[e]	0.38[e]	4.36[f]	0.260	< 0.01
Isobutyrate[d]	0.76[e]	0.54[f]	1.04[g]	0.054	< 0.01
Isovalerate[d]	0.69[e]	0.38[f]	0.72[e]	0.050	< 0.01

a VFA = volatile fatty acids; NH_3 = ammonia.
b Standard error of the mean (n = 4).
c Probability of a greater F-value for overall treatment F-test.
d Time x treatment interactions were significant (P < 0.05). However, general effects among treatments across time were consistent, and thus means across time are reported.
e, f, g Means within a row with different superscripts differ (P < 0.10).

Table 6. Influence of supplementation with starch or fiber to steers grazing summer fescue on ruminal fermentation characteristics (Experiment 2).[a]

	Treatment				
Item	**Control**	**Fiber**	**Starch**	**SEM[a]**	**P Value[b]**
VFA concentration, mM[d]	55.5[e]	54.2[e]	66.7[f]	3.28	< 0.01
Acetate:Propionate[d]	5.33	5.51	5.86	0.197	0.17
NH_3, mM[d]	5.7[e]	1.7[f]	1.0[f]	0.27	< 0.01
pH[d]	6.62[e]	6.52[e]	6.28[f]	0.098	0.04
	moles/100 moles				
Acetate	76.6[e]	77.6[e]	75.2[f]	0.52	< 0.01
Propionate[d]	14.4[e]	14.2[e]	13.1[f]	0.36	0.04
Butyrate[d]	6.8[e]	6.7[e]	9.9[f]	0.37	< 0.01
Valerate	0.57[e]	0.43[f]	0.53[e]	0.026	< 0.01
Isobutyrate	0.77[e]	0.59[f]	0.64[f]	0.031	< 0.01
Isovalerate[d]	0.79[e]	0.47[f]	0.60[f]	0.055	< 0.01

a VFA = volatile fatty acids; NH_3 = ammonia.
b Standard error of the mean (n = 4).
c Probability of a greater F-value for overall treatment F-test.
d Time x treatment interactions were significant (P < 0.05). However, general effects among treatments across time were consistent, and thus means across time are reported.
e, f, g Means within a row with different superscripts differ (P < 0.10).

Effects of Season and Sampling Method on Measures of Forage Quality in Fescue-Based Pastures

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Introduction

In the southeastern United States, tall fescue is an important component of diets for grazing cattle. Our ability to optimally use this forage base is limited because of imperfect information on forage availability and quality at any point in time. This is a consequence of the many factors influencing forage quality, including forage type, season, and method of sample collection. Also, little information exists regarding ruminal degradability of protein in tall fescue, and current feeding systems require estimates of degradable intake protein. Limitations in the use of traditional approaches (e.g., *in situ*) for estimating degradable intake protein have spawned interested in rapid enzymatic assays to generate these estimates. However, these approaches must be validated on a wide variety of forages before becoming generally accepted.

One means for producers to obtain information on grazed forage quality is to clip pasture samples for chemical analysis. However, it is well recognized that the quality of diet selected by grazing cattle will differ from the quality of clipped samples. The magnitude of this difference can depend on forage type, grazing management, and season.

This experiment was conducted to quantify the differences in chemical composition between hand-clipped and masticate samples at different times during the grazing season for continuously grazed fescue and fescue/red clover paddocks, and to compare protein degradability estimates of masticate samples using *in situ* and enzymatic techniques.

Procedures

Eight 0.76-ha (1.9-ac), endophyte-infected, Kentucky 31 tall fescue paddocks were used in a season-long grazing trial. Four paddocks, selected at random, were fertilized with 56 kg N/ha (50 lb N/ac) in March 2000. The remaining four paddocks were interseeded with red clover at a rate of 5.6 kg/ha (5.0 lb/ac) during March 2000, then fertilized with 21 kg K/ha (19 lb K/ac) and 2 kg B/ha (1.8 lb B/ac). Paddocks were grazed continuously by beef steers (average body weight = 288 kg; 635 lb) at an average season-long stocking rate of 970 kg/ha (866 lb/ac).

From April 23, 2000, through October 9, 2000, hand-clipped and ruminal masticate samples were collected at 28-day intervals from each paddock. Six hand-clipped samples were taken from each paddock using a 0.25-square-meter quadrat. Within each collection period, botanical composition was estimated by manually separating 10 clipped samples into tall fescue, red clover, other grasses, weeds, and senescent material. Samples were dried for 96 hours in a 55°C forced-air oven and ground through a 1-mm screen with a Wiley mill.

Four ruminally cannulated crossbred steers (average body weight = 288 kg; 635 lb) were used to collect masticate samples. Each paddock was sampled once in the morning and once in the evening over the course of two days. Masticate samples were freeze dried and ground through a Wiley mill.

All hand-clipped and ruminal masticate samples were analyzed in duplicate for dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and protein degradability. Estimates of protein degradability for all samples were generated using a 48-hour incubation in 0.33 activity units of *Streptomyces griseus* protease. Additionally, protein degradability estimates of masticate samples were generated with *in situ* techniques for comparison with the enzymatic approach. For *in situ* measures, we incubated samples in Dacron bags in the rumen of each of four ruminally cannulated crossbred steers (360 kg; 794 lb) that were fed a 70:30 forage:concentrate diet at 1.5% of body weight twice daily. Bags were either unincubated (0 hours) or incubated in the rumen for 6 or 96 hours. All bags, including 0-hour bags, were rinsed using five rinse cycles (1 minute agitation and 2 minutes spin per rinse) in a top-loading washing machine. After rinsing, all bags were dried at 55°C and residual nitrogen was measured. Microbial contamination of residues was corrected using purines as a microbial marker.

Data were analyzed using a model for repeated measures within a split-plot design with a completely randomized whole-plot by the MIXED procedure of SAS. Forage type, sampling method, and month were included as fixed effects and paddock (forage type) as a random effect. Orthogonal polynomial contrasts were used to identify linear, quadratic, and cubic time effects.

Results and Discussion

Standing forage biomass was somewhat greater in fescue than in fescue/red clover pastures from April ($P < 0.05$) through June ($P < 0.05$; Figure 1). No differences ($P > 0.10$) were detected between the two forage types after June. As is typical for cool-season grasses in this region, our peak in forage biomass occurred in May and declined during the summer months (cubic; $P < 0.01$). Because pastures were continuously stocked, we did not observe a resurgence in standing forage biomass in the fall.

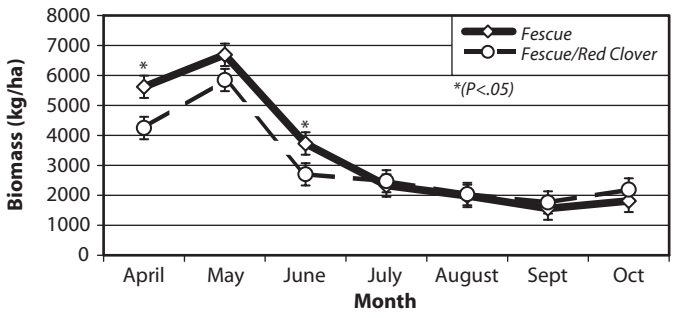
Interseeding red clover into fescue paddocks only increased ($P = 0.03$) the proportion of red clover from an average of 1% to an average of 7% of the standing forage biomass dry weight (Figure 2). No differences due to forage type ($P > 0.10$) were detected in the proportion of any of the other measured botanical components.

The major seasonal effect detected was an increase in the proportion of the biomass comprised of senescent material through July, followed by a decrease through October (quadratic; $P < 0.01$). Because of the poor stand establishment for red clover, very little difference was observed in chemical composition of paddocks with or without interseeded red clover. Forage type x sampling method x month interactions were not detected ($P > 0.10$) for any of the chemical constituents evaluated (data not shown). Forage type x month interactions were detected ($P < 0.10$) for crude protein concentration and protein degradability (estimated with the *Streptomyces griseus* protease procedure). However, the magnitude of differences between forage types was small. For crude protein, differences ($P < 0.10$) were only detected for samples collected during the months of April, August, and September (data not shown), when the fescue/red clover paddocks had from 1.5% less to 1.0% more crude protein than the fescue paddocks. For protein degradability, differences ($P < 0.10$) were only detected for September and October samples (data not shown), when the maximum difference in protein degradability was a 2% greater degradable intake protein value for samples from the fescue/red clover paddocks.

Except for degradable intake protein estimates, the forage type x sampling method interaction was nonsignificant and the main effect of forage type was nonsignificant for any of the forage quality variables.

Because of the minimal influence of forage type on measurements of forage quality, data summarized in Figures 3 through 8 were averaged across forage type, and emphasis was

Figure 1. Effect of forage type on standing forage biomass. Cubic response ($P < 0.01$) for both fescue and fescue/red clover.



placed on differences due to season and sampling methods.

Organic matter of ruminal masticate samples increased from April to May and was fairly constant afterward (cubic; $P < 0.10$). Masticate OM concentrations were lower ($P < 0.10$) throughout the grazing season than OM of hand-clipped samples, which remained fairly steady across months (quadratic; $P < 0.01$; Figure 3). The average difference of about 5.0 percentage units OM between clipped and masticate samples was attributed to salivary ash contamination. To account for this difference, all other constituents have been expressed on an OM basis.

On average, masticate samples had 4.5 percentage units higher CP concentrations from April to September as compared to clipped samples, and both responded cubically ($P < 0.01$) across time, with maximum values in April and minimum values in June (Figure 4). No difference ($P > 0.10$) was seen between clipped and masticate CP concentrations during October. This lack of difference could be attributed to low forage availability that could have inhibited selectivity.

On average, clipped samples contained 5.5 percentage units greater NDF concentrations from April to September ($P < 0.10$) than masticate samples, whereas no difference ($P > 0.10$) existed in October (Figure 5). For both clipped (quadratic; $P < 0.01$) and masticate (cubic; $P < 0.01$) samples, NDF concentrations peaked in midsummer, coinciding with a peak in the proportion of mature, senescent forage in the paddock. Concentrations of ADF (Figure 6) followed the same general trends seen with NDF concentrations. Clipped samples contained on average 3.0 percentage units greater ($P < 0.01$) ADF from April-September than masticate samples. As seen with CP, ADF and NDF concentrations of clipped and masticate samples did not differ ($P > 0.10$) in October.

Forage type and sample collection method interacted ($P < 0.01$) for *S. griseus* protease protein degradation estimates, but differences due to forage type were minimal when compared to differences seen between sample collection methods. For fescue paddocks, degradable intake protein estimates averaged 59.4% and 71.2% of CP, whereas for fescue/red clover paddocks, estimates averaged 61.4% and 70.6% of CP for clipped and masticate samples, respectively. Protein degradability of masticate samples (Figure 7) were lower in June than in other months (cubic; $P < 0.01$), and were, on average, 10.5 percentage units greater ($P < 0.01$) across the grazing season than in hand-clipped samples, which were at their lowest level during August (quadratic; $P < 0.01$).

Unlike the other constituents in which differences between clipped and masticate samples were fairly consistent across months, differences in *S. griseus* degradable intake protein varied from 4 percentage units in May to 20 percentage units in August. This variation across months makes it difficult to make assumptions for protein degradability of diets selected by cattle based on clipped samples. On average, across the entire grazing season, 60% of the total CP in clipped samples and 70% of the total CP in masticate samples were estimated to be ruminally degradable.

Figure 2. Relative proportions (wt. of DM) of biomass comprised of selected forage components in fescue (a) and fescue/red clover (b) paddocks.

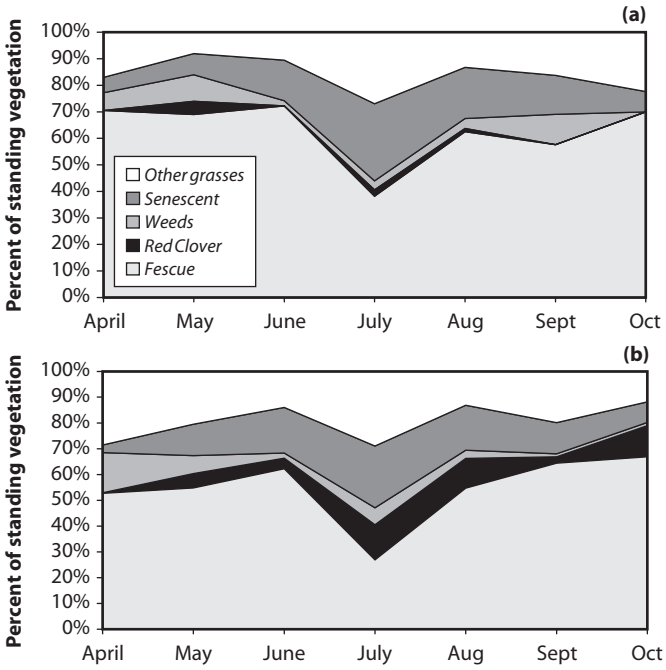


Figure 3. Effects of forage collection method on organic matter concentrations. Sampling method x time interaction ($P < 0.01$). Quadratic response for clipped ($P < 0.01$), and cubic response for masticate ($P < 0.01$).

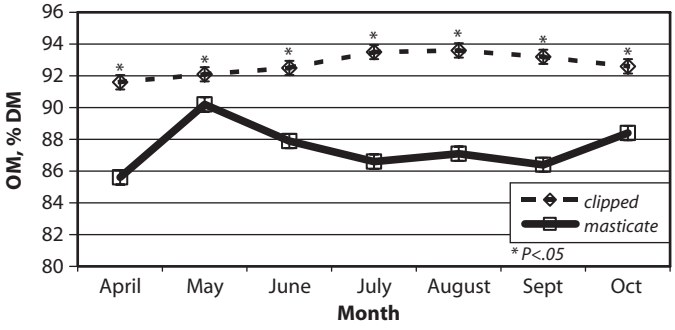


Figure 4. Effects of forage collection method on crude protein concentrations. Sampling method x time interaction ($P < 0.01$). Cubic response for both clipped and masticate ($P < 0.01$).

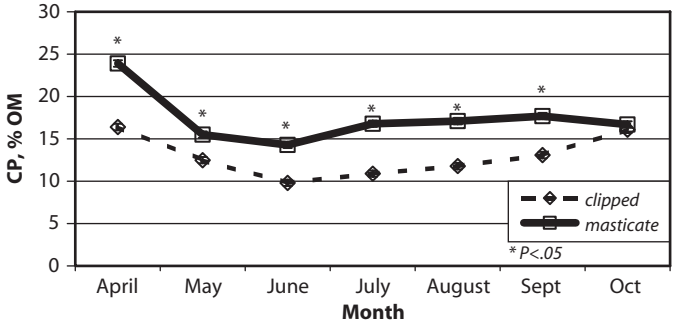


Figure 5. Effects of forage collection method on neutral detergent fiber concentration. Sampling method x time interaction (P < 0.01). Quadratic response for clipped (P < 0.01), and cubic response for masticate (P < 0.01).

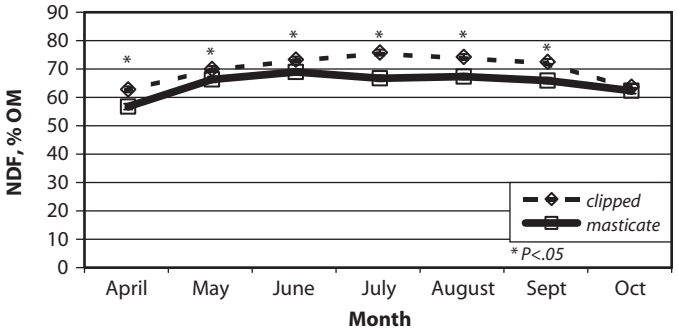
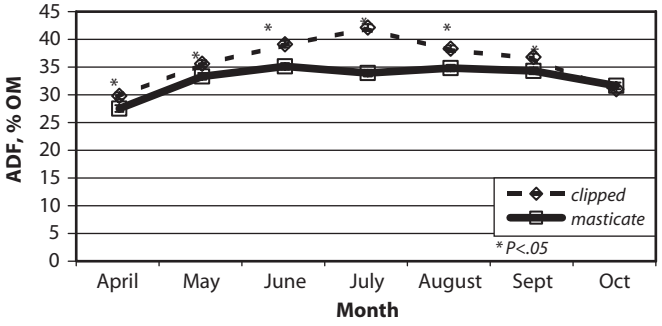


Figure 6. Effects of forage collection method on acid detergent fiber concentration. Sampling method x time interaction (P < 0.01). Quadratic response for clipped (P < 0.01), and cubic response for masticate (P < 0.01).



Part of this difference may be attributed to differences in drying techniques. It is recognized that oven drying, as was practiced with the clipped samples, will result in decreased estimates of protein degradability, as compared with freeze drying, as was done with masticate samples. However, values should represent differences between "standard" approaches for obtaining forage samples (which would include oven drying) and our best estimate of diets consumed by grazing animals (which incorporates freeze drying).

Comparison of In Situ versus In Vitro Techniques

Comparison of *in situ* and *S. griseus* protease procedures are shown in Figure 8. A technique x time interaction existed (P < 0.01) in which no differences (P > 0.10) were seen between the two procedures early in the season (April to June), whereas from July to October the *S. griseus* protease procedure gave larger (P < 0.01) estimates of degradable intake protein than the *in situ* procedure. Even though differences were seen late in the season, the *S. griseus* protease procedure did seem to mimic the trends of the *in situ* procedure. The overall average difference between the two procedures across the entire grazing season was only 3.0% degradable intake protein (percentage of CP). Procedural differences with respect to incubation time, maintenance of rumen-fistulated animals, and applicability to commercial laboratories favor the *S. griseus* protease over the *in situ* procedure.

Summary

Information describing effects of seasonal changes and sampling methods on measurements of forage quality is limited for fescue-based pastures. Eight continuously grazed, 0.76-ha, fescue-based paddocks were used to compare forage type, method of collection, and seasonal effects on forage quality. Four paddocks were interseeded with red clover. Ruminal masticate and hand-clipped samples were collected every 28 days from April until October. We had poor establishment of red clover, and thus its presence did not influence quality of diet selected by grazing steers.

Concentrations of OM, NDF, and ADF were greater for clipped samples than for masticate samples. CP and protein degradability values were lower for clipped samples than for masticate samples. We also compared two methods for measuring protein degradability and found that a rapid enzymatic procedure (using *Streptomyces griseus* protease) provided protein degradability estimates that compared favorably with *in situ* procedures for masticate from fescue-based pastures.

Figure 7. Effects of forage collection method on *Streptomyces griseus* degradable intake protein (SGDIP) concentration. Sampling method x time interaction (P < 0.01). Quadratic response for clipped (P < 0.01), and cubic response for masticate (P < 0.01).

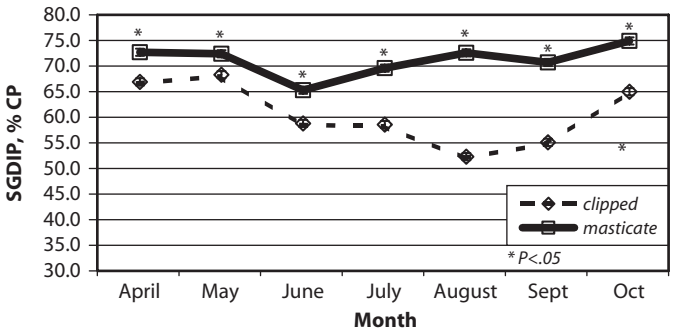
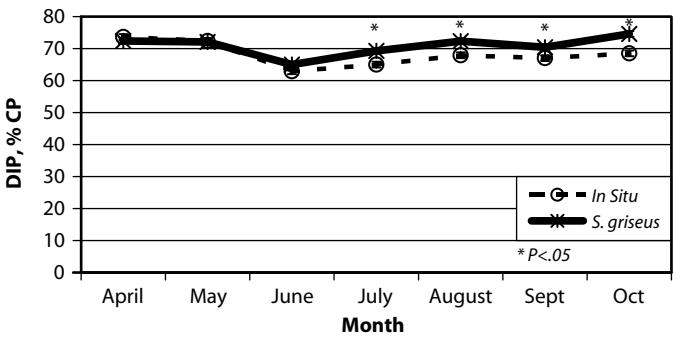


Figure 8. Effects of analysis procedure on degradable intake protein (DIP) concentration. Sampling method x time interaction (P < 0.01). Cubic response for *in situ* procedure (P = 0.01), and quadratic response for *S. griseus* procedure (P < 0.01).



Evaluation of Corn and Soybean Hull Supplements on Performance of Stocker Cattle Grazing Endophyte-Infected Fescue

B.G. Fieser, E.S. Vanzant, and L.J. Driedger

Introduction

Cattle grazing only forages generally do not consume sufficient nutrients to meet their genetic potential for growth. Research information suggests that energy, rather than protein, limits cattle growth when grazing properly managed tall fescue pastures. Therefore, supplemental energy is often incorporated into the diet to balance nutrients and increase gains.

Carbohydrate-based energy supplements can typically be broken into two classes: fiber- or starch-based. Fiber-based energy supplements are commonly by-product feedstuffs rich in highly digestible fiber such as soybean hulls, corn gluten feed, and beet pulp. Cereal grains are the most common source of starch-based energy supplements, including corn, sorghum, and barley. Starch-based supplements have high energy values but have been shown to have the potential to decrease forage digestion.

Fiber-based supplements do not exhibit such effects and, therefore, can have feeding values similar to grains when fed as supplements to grazing animals. However, the relative differences observed between starch- and fiber-based supplements may be related to forage quality and other factors that change during the course of the grazing season. This experiment was conducted to evaluate growth responses at different times of the year by stocker steers grazing endophyte-infected fescue and supplemented with corn or soybean hulls.

Procedures

Grazing studies were conducted on eight 6.1-ha (15-ac), endophyte-infected, tall fescue pastures at the University of Kentucky Woodford County Animal Research Center from April 2000 through September 2001. In the first study (April to September 2000), 160 crossbred beef steers (average initial weight = 242 kg; 534 lb) were used. In the second study, conducted on stockpiled fescue in January and February 2001, we used 80 crossbred beef steers (average initial weight = 290 kg; 639 lb), and in the summer grazing season of 2001 (May to September), we used 128 crossbred beef steers (average initial weight = 272 kg; 600 lb).

In order to match stocking rates to forage growth profiles in the summer phases, steers were confined to one-half of each pasture during the early season (until late June), resulting in early-season stocking rates of 1,830 (Experiment 1) and 1,740 kg/ha (Experiment 2). In Experiment 1, hay was cut from the other half of each pasture in late May. At the end of June in both years, steers were allowed access to the entire pastures, and, in Year 1, steer numbers were reduced by one-fourth during the late season, resulting in late-season stocking rates of 1,000 kg/ha in both years.

Pastures were clipped once in June of each year at a height of approximately 20 cm to remove seed heads. Pastures were fertilized with 56 kg N/ha in both March and June. For the stockpiled grazing study, pastures received 56 kg N/ha in September and were stocked at 1,150 kg/ha from January through March (56 days).

In all studies, steers had free-choice access to water and a mineral supplement. Each study was divided into 28-day periods, resulting in six periods in the first grazing study, two in the second, and five in the third. In each period, steers in two randomly assigned pastures received no additional supplement, while steers in each of three randomly assigned pastures received cracked corn, and steers in the remaining three pastures received pelleted soybean hulls. Supplements were group-fed in feed bunks at a rate of 0.75% of body weight (as-fed basis) each day at approximately 0700 to 0900.

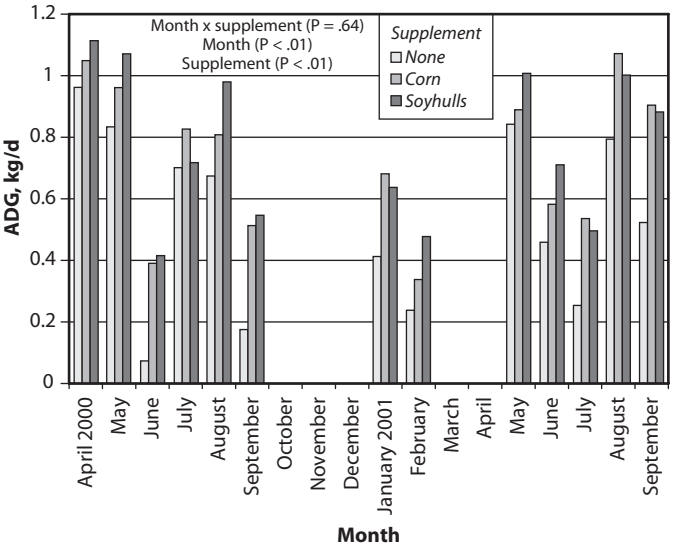
Cattle were weighed at the end of each 28-day period following an overnight stand without access to feed or water. In order to test for period x treatment interactions without carryover effects from supplementation treatments, at the end of each period steers were re-allotted to experimental pastures in such a way as to balance the number of steers in a pasture group that had previously been on each of the treatments. In addition, pasture treatments were reassigned at that time.

Data were analyzed using the MIXED procedure of SAS with a model appropriate for repeated measures within a completely randomized design. Pastures were designated as the experimental unit on which repeated measures were made. Treatment means were separated using a protected ($P < 0.10$) Fisher's LSD.

Results and Discussion

Steer gains are shown in Figure 1. Despite some numerical differences in response to supplements during different months, no month x supplement interaction was detected ($P = 0.64$). In general, gains were lowest during midsummer (June 2000 and

Figure 1. Average daily gain (ADG) of steers receiving no supplement, or cracked corn or soybean hulls at 0.75% of body weight during each period of the experiment. Refer to text for listing of average responses for each treatment.



July 2001), as is common on endophyte-infected fescue. Low gains during these times are a function of increased plant maturity and of decreased intake in response to heat stress, which is exacerbated by the presence of the endophyte.

In 2000, average monthly minimum and maximum temperatures increased from April through June and remained fairly constant until August (Figure 2). In 2001, temperatures increased through August. Differences in temperature patterns, as well as potential differences in time of peak alkaloid levels from the endophyte between the two years, likely explain differences in cattle growth patterns during the summers of the two years.

It might be expected that there would be greater potential for cattle to respond to supplementation when forage quality and intake supported lower levels of gain. In order to evaluate this, we conducted regression analysis, comparing the response to supplementation within a period (averaged between corn and soybean hull groups) to the gain achieved without supplementation (Figure 3).

Results support the hypothesis that supplementation responses are greater during times in which cattle growth without supplementation is low. However, nonsupplemented gain accounted for only about 40% of the variation in response to energy supplements, indicating that other factors, some possibly related to forage composition, are quite important. Across the entire study, average daily gains averaged 0.54, 0.73, and 0.77 kg/day (1.18, 1.62, and 1.70 lb/day) for the non-supplement, corn, and soybean hull groups, respectively. There was an increase ($P < 0.01$) in cattle gain in response to supplementation; however, gains by steers supplemented with soybean hulls tended ($P = 0.12$) to be greater than for those receiving cracked corn.

In order to help evaluate the economic potential of supplementation, we calculated partial conversion efficiencies, which indicate the kg (or lb) of supplement consumed per kg (or lb) of additional gain (Table 1). It is important to note that these values were determined with supplementation at 0.75% of body weight (which would be 4.5 lb of supplement for a 600-lb steer).

In general, partial conversion efficiencies of supplementation are improved (lower values) with lower levels of supplementation. Partial conversion efficiencies in the neighborhood of 10 to 1 are common with energy supplementation on a variety of forages. With the exception of July 2000, when we obtained essentially no additional gain with the soybean hulls, the partial conversion efficiencies for soybean hulls remained at or below 12.4. With corn, however, these efficiencies exceeded 15 in 7 of the 13 periods of this study. Thus, not only was the average partial conversion efficiency better for soybean hulls, the likelihood of obtaining economic benefit from soybean hull supplementation was greater as well.

This information can be used with supplement pricing information to compare the value of supplementation against the value of the added gain, as shown in Table 2. For example, with a partial conversion efficiency of 10.0 and a supplement cost of $80/ton, it costs $0.40 for each additional pound of gain. When working with the supplement costs, it is important to include the actual cost of supplement delivered to the bunk, which will include processing, delivery, labor, and fuel costs. Thus, one can see that unless supplement prices are very inexpensive and/or cattle prices are quite high, it is highly difficult to reap economic value in the form of additional cattle weight from supplementation when partial conversion efficiencies exceed 15:1.

Summary

Studies were conducted across multiple grazing seasons (one winter and two summer seasons) on endophyte-infected tall fescue. Supplementation with cracked corn or pelleted soybean hulls at 0.75% of body weight improved ($P < 0.05$) steer daily gains by an average of 0.21 kg/day (0.46 lb/day) compared to steers receiving no energy supplementation. Gains with soybean hulls (0.77 kg/day; 1.70 lb/day) tended ($P = 0.12$) to be greater than gains with cracked corn (0.73 kg/day; 1.62 lb/day). Statistical analysis suggested that responses to supplements were

Figure 2. Precipitation and maximum and minimum temperatures at the University of Kentucky Animal Research Center in Woodford County, Kentucky, during each period of the experiment.

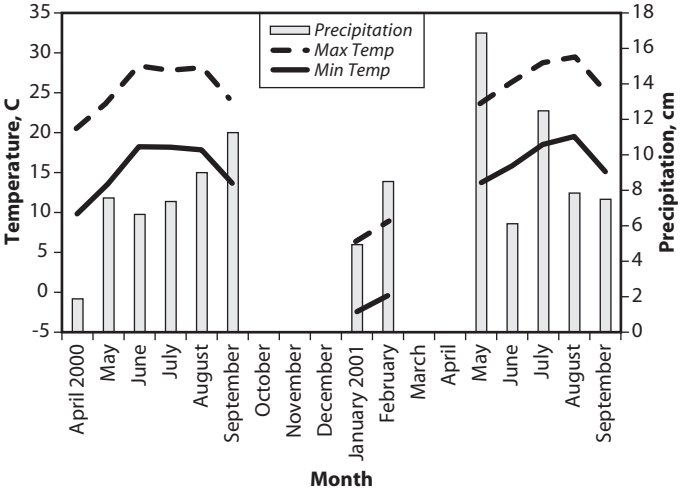
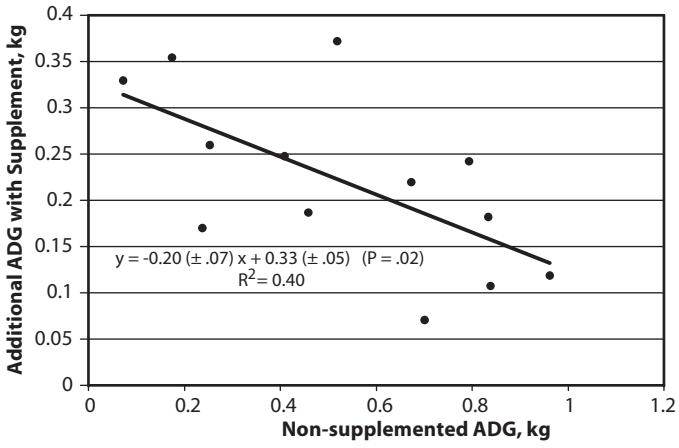


Figure 3. Additional gain from supplementation as related to average daily gain without supplement.



unrelated to the time of year (supplement x month interaction; P = 0.64), although the magnitude of gain increase with supplementation showed a weak (R^2 = 0.40; P = 0.02) negative relationship with unsupplemented steer gains. Partial conversion efficiencies averaged 11.3 and 9.5 kg supplement/kg additional gain for corn and soybean hulls, respectively. These data indicate that both corn and soybean hulls have potential to be economically viable supplements for growing cattle grazing endophyte-infected fescue and that the likelihood of obtaining reasonable supplement conversion efficiencies may be greater with soybean hulls.

Table 1. Partial conversion efficiencies for corn and soybean hulls offered at 0.75% of body weight during each month of the experiment, kg of supplement (as-fed basis)/kg of additional gain over unsupplemented cattle.

Date	Corn	Soybean Hulls
April 2000	20.8	12.0
May 2000	16.0	8.6
June 2000	7.1	6.5
July 2000	18.3	138.2
August 2000	17.7	7.8
September 2000	7.3	6.6
January 2001	8.1	9.8
February 2001	23.0	9.6
May 2001	40.6	12.4
June 2001	18.2	8.9
July 2001	8.4	9.9
August 2001	8.9	12.0
September 2001	7.0	7.4
Average[a]	**11.3**	**9.5**

[a] This average has been calculated as the average supplement quantity fed divided by the average gain response above control for each group and thus does not equal the arithmetical mean of the numbers listed above (the ratio of averages does not equal the average of ratios).

Table 2. Supplement cost per pound of additional gain at various supplement prices and partial conversion efficiencies.[a]

Supplement Price		Partial Conversion Efficiency							
$/Ton	$/Lb	5	6	7	8	9	10	15	20
$60.00	$0.030	0.15	0.18	0.21	0.24	0.27	0.30	0.45	0.60
$70.00	$0.035	0.18	0.21	0.25	0.28	0.32	0.35	0.53	0.70
$80.00	$0.040	0.20	0.24	0.28	0.32	0.36	0.40	0.60	0.80
$ 90.00	$0.045	0.23	0.27	0.32	0.36	0.41	0.45	0.68	0.90
$100.00	$0.050	0.25	0.30	0.35	0.40	0.45	0.50	0.75	1.00
$110.00	$0.055	0.28	0.33	0.39	0.44	0.50	0.55	0.83	1.10
$120.00	$0.060	0.30	0.36	0.42	0.48	0.54	0.60	0.90	1.20
$130.00	$0.065	0.33	0.39	0.46	0.52	0.59	0.65	0.98	1.30
$140.00	$0.070	0.35	0.42	0.49	0.56	0.63	0.70	1.05	1.40
$150.00	$0.075	0.38	0.45	0.53	0.60	0.68	0.75	1.13	1.50
$160.00	$0.080	0.40	0.48	0.56	0.64	0.72	0.80	1.20	1.60
$170.00	$0.085	0.43	0.51	0.60	0.68	0.77	0.85	1.28	1.70
$180.00	$0.090	0.45	0.54	0.63	0.72	0.81	0.90	1.35	1.80
$190.00	$0.095	0.48	0.57	0.67	0.76	0.86	0.95	1.43	1.90
$200.00	$0.100	0.50	0.60	0.70	0.80	0.90	1.00	1.50	2.00

[a] Partial conversion efficiency is pound of supplement per pound of additional gain.

Alternative Supplements for Cattle Consuming Corn-Silage Diets: Effects on Digestion and Ruminal Fermentation

C.M. Howlett, E.S. Vanzant, B.G. Fieser, R.F. Bapst, and K.B. Combs

Introduction

Source of dietary energy, irrespective of energy level, can have important effects on reproductive function of beef females. Additionally, it is well recognized that source of energy can affect ruminal fermentation and digestion of forage-based diets. For example, both starch and fat have been shown to decrease fiber digestion when added to high-forage diets. Furthermore, some oilseeds contain additional anti-quality factors (e.g., enzyme inhibitors in soybeans) that could have effects on feed utilization. Limited information is available to describe such effects with corn-silage diets. The objective of this experiment was to evaluate the influence of various energy supplements, including starch, fat, and fiber-containing feeds, on digestion and ruminal fermentation of corn-silage diets by beef cattle.

Procedures

Six crossbred steers (387 kg; 853 lb) with ruminal and duodenal cannulas were used in a six-by-six Latin square design to evaluate supplemental energy source effects on utilization of corn-silage diets. Dietary treatments consisted of a corn-silage-(CP = 9.1%; TDN = 67%) based diet containing:

- soybean meal at 10% of the dry matter intake,
- corn and soybean meal at 56% of the dry matter intake,
- whole linted cottonseed at 15% of the dry matter intake,
- whole raw soybeans at 15% of the dry matter intake,
- whole raw soybeans at 25% of the dry matter intake, or
- pelleted soybean hulls at 30% of the dry matter intake (Table 1).

Diets were formulated to have equal protein concentrations (13.7% CP) except for the 25% soybean treatment (17% CP) and were fed twice daily at 1.8 x NE_m based on formulated energy values. We selected these treatments to mimic those used in a companion study to evaluate effects on heifer growth and reproductive performance (Diets 2, 3, 4, and 6) and to evaluate effects of a higher inclusion of oilseed (Diet 5). Additionally, a control diet (Diet 1) consisting of silage (and soybean meal to

Table 1. Composition of experimental diets.[a]

	Treatment					
Feedstuff	**Soybean Meal (SBM)**	**Corn/SBM**	**Cottonseed**	**15% Soybeans**	**25% Soybeans**	**Soyhulls**
	Ingredient Composition, % of DM					
Corn silage	88.5%	42.0%	42.0%	42.0%	42.0%	42.0%
Corn	0.0%	46.8%	35.5%	41.3%	31.3%	17.8%
Soybean meal	10.0 %	9.5%	5.8%	0.0%	0.0%	8.5%
Whole cottonseed	0.0%	0.0%	15.0%	0.0%	0.0%	0.0%
Whole soybeans	0.0%	0.0%	0.0%	15.0%	25.0%	0.0%
Soyhulls	0.0%	0.0%	0.0%	0.0%	0.0%	30.0%
Mineral	1.5%	1.7%	1.7%	1.7%	1.7%	1.7%
	Chemical Composition, % of DM					
OM	94.1	95.2	94.9	95.0	93.8	94.0
CP	13.2	13.7	13.8	13.7	16.9	13.3
NDF	44.1	27.1	32.0	27.8	28.3	43.4
ADF	22.8	12.6	17.5	13.1	13.6	25.7
EE	3.3	3.3	5.3	4.9	9.7	2.5

[a] DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract.

equalize protein with other diets) was included to provide a baseline for comparing other diets.

Steers were maintained in individual pens for 21 days within each period of the Latin square. They were adapted to diets for 10 days. Total feces were collected and subsampled on Days 11 through 16, followed by duodenal sampling on Days 17 through 19. On Day 20, ruminal fluid samples were collected every three hours for 12 hours for characterizing ruminal pH and VFA and ammonia concentrations. Feed and fecal samples were dried for 48 hours at 55°C in a forced-air oven to determine dry matter concentrations. Dry matter intake and fecal output were used to calculate total-tract dry matter digestibilities.

Data were analyzed using the MIXED procedure of SAS. Dietary treatment and period were included as fixed effects, and steer was included as a random effect in the model. Ruminal fermentation characteristics were analyzed with a model appropriate for repeated measures within a Latin square. Although time x treatment interactions were significant for many of the ruminal fermentation variables, overall treatment effects were fairly consistent at different sampling times. Thus, only main effects have been shown. Treatment means were separated using a protected Fisher's LSD.

Results and Discussion

Dry matter digestibility was lowest ($P < 0.10$) for Diet 1, which contained only supplemental soybean meal and no additional energy supplement, greatest for diets with corn/SBM or soybean hulls, and intermediate for diets containing oilseeds. Using a summative equation to estimate the energy value of the corn silage for formulation purposes, we had estimated a digestibility value of 67%. Using this value and the book value for the digestibility of soybean meal (84%; NRC, 1996), the measured digestibility of the total diet was accurately predicted (predicted value = 67.7%; observed value = 67.5%). Similar calculations were performed with each of the other diets to indicate possible positive or negative associative effects.

With corn addition at 47% of the diet (Diet 2), we had little indication of associative effects (actual - predicted value = 1.4 percentage units). With oilseed supplements, measured digestibility values were below predicted values, suggestive of negative associative effects. The difference between actual and predicted values was -5.2, -3.8, and -5.9 percentage units for Diets 3, 4, and 5, respectively. Thus, cottonseeds appeared to depress forage digestibility to a greater extent than soybeans when both were included at 15% of the diet, and increasing soybeans to 25% of the diet appeared to have additional negative effects on forage digestion. The difference between actual and predicted digestibility with soybean hulls was +3.9 percentage units, suggestive of a positive effect on forage digestion. Other research has suggested that highly digestible fiber supplements, such as soybean hulls, can have a positive influence on digestion of forage-based diets.

Ruminal ammonia concentrations were greater ($P < 0.10$) for 25% whole soybeans than for other treatments. This was expected because ruminal ammonia levels have a strong positive association with dietary crude protein content, and our 25% soybean diet had greater crude protein than the other treatments. Ruminal ammonia levels on all treatments exceeded values typically considered to be sufficient for microbial growth on forage-based diets.

Ruminal pH was lowest for the two diets with the greatest digestibilities (corn/soybean meal and soybean hulls). Ruminal pH did not differ among the other diets. Greater digestibility is expected to result in greater acid production within the rumen and thus to lower pH. Although ruminal pH in the proximity of 6.2 is considered to potentially hamper forage digestion, there was no evidence that forage digestion was hindered on these two diets, as discussed above.

High levels of fiber digestion typically support high proportions of acetate production in the rumen. Accordingly, our greatest acetate proportions were detected for the control diet (Diet 1) and the soybean hull diet (Diet 6), which contained the greatest

Table 2. Influence of supplementation of corn silage diets on dry matter digestibility and ruminal fermentation characteristics.

	Treatment						
Item	Soybean Meal (SBM)	Corn/SBM	Cottonseed	15% Soybeans	25% Soybeans	Soyhulls	SEM[a]
DMD[b]	67.4[c]	79.6[e]	73.3[d]	75.6[d]	73.9[d]	78.3[e]	1.21
Acetate:Propionate	4.2[de]	3.8[d]	3.2[c]	3.2[c]	2.9[c]	4.4[e]	.25
NH_3 mM	6.9[c]	7.1[c]	6.2[c]	7.0[c]	11.3[d]	6.8[c]	.65
pH	6.51[d]	6.23[c]	6.46[d]	6.49[d]	6.45[d]	6.27[c]	.079
	moles/100 moles						
Acetate	67.6[e]	64.0[d]	64.0[d]	63.0[cd]	61.4[c]	67.1[e]	1.16
Propionate	16.8[c]	17.3[c]	20.6[d]	20.0[d]	21.6[d]	16.5[c]	1.04
Butyrate	11.5[cd]	14.4[e]	10.5[c]	11.4[cd]	10.9[c]	12.9[de]	.70
Valerate	1.1[c]	1.2[cd]	1.2[cd]	1.3[d]	1.5[e]	1.1[c]	.06
Isobutyrate	1.1[d]	1.1[d]	1.2[e]	1.5[f]	1.6[f]	0.9[c]	.05
Isovalerate	1.8[cd]	1.2[de]	2.4[e]	2.8[f]	3.1[f]	1.6[c]	.19

a Standard error of the mean (n = 6).

b DMD = total tract dry matter digestibility.

c, d, e, f Means within a row with different superscripts differ (P < 0.10).

concentrations of fiber. Acetate proportions were lowest when the high level of soybeans was fed (Diet 5), in agreement with other indications that this diet suppressed forage digestion.

Ruminal propionate proportions were greater (P < 0.10) and acetate:propionate ratios were lower for the diets containing oilseeds than for other diets. Additionally, acetate:propionate ratio was greatest with the soybean hull diet. This is in agreement with other research showing that dietary lipid supplementation increases ruminal propionate proportions. Additionally, other research has suggested that increasing ruminal propionate can have stimulatory effects on fertility and reproduction in beef females. It is possible that some of the effects on reproduction attributed to supplemental dietary fat are mediated through alterations in ruminal propionate production, although direct effects of individual fatty acids on reproductive performance have been implicated in other studies.

Ruminal proportions of butyrate were greatest (P < 0.10) when the largest amount of corn grain was fed (Diet 2) and intermediate with soybean hulls. Molar proportions of the minor VFA, valerate, isobutyrate, and isovalerate tended to be least with soybean hull supplementation, greatest with the high level of soybeans, and intermediate for the other treatments. Proportions of ruminal VFA represent a balance between production and utilization. These particular VFA serve as a nutrient source for fiber-digesting bacteria. Thus, on the diet with the greatest suspected fiber digestion, the soybean hull diet, it is likely that low proportions of the minor VFA reflected increased use by an active fiber-digesting population. Additionally, the primary substrate for production of these VFA is amino acids, which may help explain the greater levels when the high level of soybean was fed because this diet had greater total CP than the other diets.

Summary

This study indicates that supplementing corn-silage-based diets with whole oilseeds at 15 to 25% of diet dry matter may depress digestibility of the forage component of the diet. However, there were no indications of negative effects on digestion when a corn/SBM supplement was used, and incorporation of soybean hulls at 30% of the diet may enhance forage digestion. Some shifts in ruminal VFA concentrations, consistent with dietary fat and protein supply, were also apparent. Results from this study will help to more accurately characterize the feeding value of feedstuffs when used in mixed diets. Further study is under way to characterize the influence of these diets on the profile of nutrients available for absorption at the small intestine.

Influence of Abomasal Infusion of Starch Hydrolysate and/or Casein on Pancreatic Exocrine Secretion and Blood Concentrations of Pancreatic Hormones and Glucose in Beef Steers

J.A. Benson, K.C. Swanson, and D.L. Harmon

Introduction

Cereal grains provide a major source of energy for beef production in the United States. The majority of starch contained in grains is fermented in the rumen but, depending on grain type and method of processing, it has been estimated that between 4% and 60% of dietary starch reaches the small intestine in cattle fed high-concentrate diets. Starch digestion and glucose absorption in the small intestine are theoretically more efficient than ruminal fermentation; therefore, the potential to improve the energetic efficiency of production exists.

However, data suggest that there are limits to starch digestion in the small intestine of ruminants. In particular, secretion of α-amylase, the pancreatic enzyme that initiates starch breakdown, is limiting. Previous research from our group has shown that abomasal infusion of increasing levels of partially hydrolyzed starch (SH) or glucose results in linear decreases in secretion of α-amylase activity of beef steers. However, increasing the postruminal supply of protein may have beneficial effects on starch digestion. Other research from our group has shown that increasing levels of abomasal casein infusion results in improved small intestinal starch disappearance and increased α-amylase secretion.

We do not know how the presence of both starch and protein together at the small intestine might interact to affect α-amylase secretion and starch digestion. Understanding the factors regulating pancreatic digestive enzyme production and secretion will enable improved feeding regimes to be devised that enhance pancreatic secretion of α-amylase and, therefore, improve small-intestinal starch digestion efficiency. Therefore, the objectives of the current experiment were:

- to investigate the interaction of postruminal SH and postruminal casein on pancreatic enzyme secretion in beef steers, and
- to measure plasma concentrations of hormones shown to have important roles in regulating pancreatic secretion in nonruminants, in response to postruminal SH and/or casein infusions.

Procedures

Animals, Diet, and Treatment

Eight Angus steers (290 ± 8 kg initial BW) were used in a replicated, four-by-four Latin square design. Animals were surgically prepared with abomasal cannulas for infusion and pancreatic pouch-duodenal re-entrant cannulas for quantitative collection of pancreatic juice. To minimize dietary starch flow to the small intestine, steers were fed a basal diet of alfalfa cubes at 1.75% BW, supplemented with a mineral mix at 0.015% BW. This was formulated to supply 1.2 x NE_m for a steer gaining 0.2 kg/day. Daily feed allowances were divided into 12 equal portions and fed every two hours using automated feeders.

Treatments consisted of 10 days abomasal infusion of water (control), starch hydrolysate (2.66 g/kg BW/day; SH), casein (0.6 g/kg BW/day), and a mixture of the same amounts of SH and casein (SH + casein). The first three days were an adaptation period in which 25%, 50%, and 75% of the total amount were infused, respectively. Infusion periods were separated by at least 10 days. Solutions for infusion were made up to a final volume of 6,300 ml with water and infused at a rate of 250 ml/hour into the abomasum. Starch hydrolysate is raw corn starch that has been partially hydrolyzed by a heat-stable α-amylase and was used because its digestion characteristics are similar to native starch passing through the small intestine.

Sample Collection and Analysis

On Day 10 of the abomasal infusion period, pancreatic juice and jugular blood samples were collected at 30-minute intervals for six hours. Pancreatic juice was collected under continuous vacuum into ice-cooled flasks. The weight and pH of each 30-minute sample were recorded and a 10% subsample was composited and frozen at -30°C until analyzed. The remaining sample was returned to the duodenum via the re-entrant cannula.

Samples were analyzed for concentrations of total protein, α-amylase, trypsin, and chymotrypsin activities. All analyses of pancreatic juice were completed within one week of collection. One unit of enzyme activity is defined as 1 µmole product released per minute.

Blood samples were collected from a jugular catheter established the previous day. Samples were withdrawn into heparinized syringes, centrifuged, and the plasma harvested. Plasma was aliquoted into separate tubes for each analysis, and proteinase inhibitor was added to samples for hormone analysis. Plasma samples were stored at -80°C until analyzed. Insulin and glucagon concentrations were measured using double antibody radioimmunoassays. Glucose concentrations were measured using the hexokinase method. Inter- and intra-assay coefficients of variation were less than 10% for plasma analyses.

Statistical Analysis

Mean values for each animal for each infusion period were calculated for secretion rate, pH, and plasma analyses and subsequently used in the statistical analysis. Data were analyzed as a replicated four-by-four Latin square. The statistical model included effects of square, period within square, animal within square, starch and casein infusion, and their interaction. Differences were considered significant when $P \leq 0.10$.

Results and Discussion

Results for pancreatic juice and for plasma are presented in Table 1 and Table 2, respectively. There was no effect of SH, casein, or their interaction on the rate of secretion ($P \geq 0.19$), pH ($P \geq 0.36$), or protein concentration ($P \geq 0.12$) of pancreatic juice. Rate of protein secretion was similarly unaffected by infusion ($P \geq 0.31$).

Concentration of α-amylase activity (U/mL) was decreased by SH infusion (P = 0.02) and increased by casein infusion (P = 0.03), but there was no interaction (P = 0.40). α-Amylase-specific activity (U/mg protein) was increased only by casein infusion (P = 0.09). α-Amylase secretion (U/h) exhibited a SH x casein infusion interaction (P = 0.03). Casein infusion increased α-amylase secretion when infused alone but not when infused with SH (P = 0.03).

There was no effect (P ≥ 0.35) of SH or casein infusion or their interaction on trypsin concentration (U/L). Starch hydrolysate infusion caused a significant increase in trypsin specific activity (U/mg protein). This was due to a numerical increase in trypsin concentration and decrease in protein concentration. However, this was not observed when casein was infused together with SH (P = 0.1). Similarly, there was an SH x casein interaction (P = 0.08) for total trypsin secretion (U/h). Infusion of SH + casein resulted in trypsin secretion values similar to the control, although when infused individually, SH and casein each stimulated increases in secretion. Chymotrypsin concentration (U/L) also showed an SH x casein interaction (P = 0.05). Casein increased chymotrypsin concentration when infused alone; however, when SH + casein was infused, no change occurred (P = 0.05). Both chymotrypsin specific activity and secretion also exhibited a SH x casein interaction (P ≤ 0.03). Infusion of SH and casein increased specific activity and secretion values when they were infused individually but not when infused together.

Plasma insulin concentration (ng/ml) exhibited an SH x casein interaction (P = 0.09). Both SH and casein increased plasma insulin concentrations when infused alone, but no additional increase was found when they were infused together (P = 0.09). Plasma glucagon concentration (pg/ml) was decreased (P = 0.09) by SH infusion, but there was no effect of casein infusion or any interaction (P ≥ 0.21). Starch hydrolysate infusion increased (P = 0.07) plasma glucose concentration (mg/dL). There was a trend (P = 0.11) to suggest an interaction for SH x casein infusion in that glucose concentration was not increased with SH + casein infusion, compared to SH alone.

Previous work has shown that postruminal starch infusion has a negative effect on secretion of α-amylase activity in beef steers, and this may be a major factor limiting its efficiency of digestion in the small intestine. Postruminal casein infusion has been shown to increase α-amylase secretion; therefore, it was hypothesized that infusing casein with SH may have a positive effect on α-amylase secretion and, therefore, improve starch digestion postruminally.

Infusion of SH in the current experiment resulted in a significant decrease in α-amylase concentration of pancreatic juice, as has been previously observed. However, SH infusion also resulted in a numerical increase in secretion rate of pancreatic juice, and therefore, overall α-amylase secretion was maintained. This response is not typical of most data which show a decrease in α-amylase secretion with postruminal SH infusion. Abomasal casein infusion stimulated large increases in α-amylase concentration and secretion in accordance with other work. However, infusion of casein with SH failed to alleviate the negative effect of SH on α-amylase concentration, and α-amylase secretion was not different from the control.

Infusion of SH + casein also failed to have any beneficial effects on concentration, specific activity, or secretion of protease enzymes measured in this experiment. Casein infused on its own stimulated large increases in chymotrypsin concentration and secretion, which were not achieved when it was infused together with SH. Starch hydrolysate infusion also had small positive effects on trypsin and chymotrypsin secretion that were abolished when it was infused with casein.

Table 1. Effect of abomasal infusion of starch hydrolysate (SH) and/or casein on pancreatic digestive secretion in steers.

	Abomasal Infusion					P Value		
Item	Control	SH	Casein	SH + Casein	SE	SH	Casein	SH x Casein
Secretion, mL/h	57.9	78.4	69.4	62.1	10.38	0.52	0.82	0.19
pH	8.306	8.351	8.246	8.317	0.063	0.36	0.46	0.84
Protein								
mg/mL	29.7	24.1	32.2	28.3	3.00	0.12	0.27	0.77
g/h	1.60	1.65	2.13	1.63	0.267	0.39	0.35	0.31
α-Amylase								
U/mL	420	310	614	402	59.5	0.02	0.03	0.40
U/mg protein	14.2	12.5	19.6	14.5	2.06	0.11	0.09	0.42
U/h	20406	20553	43486	20462	4892	0.03	0.03	0.03
Trypsin								
U/L	2000	2210	2267	1969	271.0	0.87	0.97	0.35
U/mg protein	0.068	0.095	0.071	0.072	0.0078	0.08	0.20	0.10
U/h	110	173	148	115	26.4	0.58	0.70	0.08
Chymotrypsin								
U/L	491	498	794	515	67.6	0.06	0.03	0.05
U/mg protein	0.016	0.021	0.025	0.019	0.0019	0.76	0.13	0.02
U/h	26.6	36.0	54.8	29.9	7.22	0.28	0.14	0.03

Table 2. Effect of abomasal infusion of starch hydrolysate (SH) and/or casein on plasma hormone and metabolite concentrations in steers.

	Abomasal Infusion					P Value		
Item	Control	SH	Casein	SH + Casein	SE	SH	Casein	SH x Casein
Hormones								
Insulin, ng/mL	0.395	0.593	0.548	0.619	0.0373	0.01	0.03	0.09
Glucagon, pg/mL	95.43	86.49	103.41	93.04	5.642	0.09	0.21	0.90
Metabolites								
Glucose, mg/dL	73.31	77.47	76.57	76.83	1.156	0.07	0.27	0.11

In general, positive effects on secretion of all pancreatic enzymes measured in response to SH infusion were due largely to the numerical increase in pancreatic juice secretion observed. An increase in pancreatic juice secretion in response to abomasal SH infusion has been observed by other researchers who suggested that it may be stimulated in response to a decrease in ileal pH as a result of small-intestinal fermentation of SH.

It is unclear how SH and casein interact to regulate pancreatic secretion. The role of hormones in mediating effects of nutrients on pancreatic exocrine secretion has been widely investigated in nonruminants but has received much less attention in ruminants. In the current experiment, plasma concentrations of insulin and glucagon, two hormones that have been shown to have stimulatory and inhibitory effects, respectively, on pancreatic exocrine secretion in nonruminants, were measured. Insulin concentrations were increased by SH and casein infusions, which are a result of stimulation by glucose and amino acids. Glucagon concentration was decreased in response to the increased plasma glucose concentration observed with SH infusion.

A numerical increase in glucagon concentration with the casein infusion was also observed, which may be stimulated by the extra amino acids supplied. There appears to be no obvious correlation of these hormones with pancreatic enzyme concentration or secretion in this trial. However, there are other hormones such as pancreatic polypeptide and peptide YY that are released in response to nutrient stimulation in the small intestine and have inhibitory effects on pancreatic enzyme secretion in nonruminants which have yet to be investigated in ruminants.

The interaction of these hormones with cholecystokinin, a hormone released in response to increased protein intake in nonruminants and which has a strong stimulatory effect on protein production and secretion, may be important. Cholecystokinin may be mediating the positive responses in pancreatic enzyme secretion observed with the abomasal casein infusion in the current experiment. Other possible explanations may involve stimulation of neural signals by postruminal nutrients or a direct effect of absorbed glucose on pancreatic cells. Both abomasal and intravenous infusion of glucose have been shown to have inhibitory effects on α-amylase production and secretion in ruminants.

In the current experiment, the increased plasma glucose in response to SH infusion may have had a negative effect on pancreatic α-amylase concentration. However, plasma glucose concentrations were similar for infusions of casein and SH + casein. Therefore, a role does not seem obvious. Clearly, regulation of pancreatic enzyme secretion in ruminants is a complex process, and further research is required before improvements in postruminal starch digestion can be made.

Summary

Improvements in postruminal digestion of starch may increase the efficiency of beef production. However, previous research has indicated that pancreatic α-amylase secretion, necessary for digesting starch in the small intestine, is decreased with increasing postruminal starch. This experiment was conducted to investigate the effects of postruminal protein (casein), which has been shown to stimulate α-amylase secretion and increase small-intestinal starch digestion, and postruminal starch on pancreatic enzyme secretion. Concentrations of hormones that may have roles in regulating pancreatic enzyme secretion were also measured.

Eight cannulated Angus steers (290 ± 8 kg initial BW) were used in a replicated four-by-four Latin square. Treatments consisted of 10 days abomasal infusion of water (control), starch hydrolysate (2.66 g/kg BW/d; SH), casein (0.6 g/kg BW/d) and a mixture of SH and casein (SH + casein). The first three days were an adaptation period. Results indicated that infusion of SH + casein failed to overcome the inhibitory effects of SH on α-amylase concentration of pancreatic juice and failed to increase α-amylase secretion, protease secretion, or plasma glucose concentration. Plasma glucose concentration was increased only in response to SH infusion. Postruminal SH or increased glucose concentrations result in strong inhibitory hormonal and/or neural signals. Plasma insulin and glucagon concentrations did not appear to have a direct role in regulating pancreatic enzyme secretion in this experiment.

Postruminal Infusion of Casein Increases Pancreatic Exocrine Secretion in Steers

C.J. Richards, K.C. Swanson, S.J. Lewis, D.L. Harmon, and G.B. Huntington

Introduction

Diet type and amount of intake affect ruminal fermentation and subsequent supply of starch to the small intestine. Today's cattle-finishing industry relies on starch in grains to supply the majority of dietary energy. At high diet intakes, 500 to 2,000 g of starch can flow to the small intestine of beef steers with an intestinal digestibility ranging from 47% to 88%. Starch digestion in the small intestine is theoretically more energetically efficient than ruminal fermentation.

However, shifting digestion to the small intestine has not commonly resulted in increased efficiency of growth. This suggests that small-intestinal starch assimilation is limited in the ruminant. It has been proposed that inadequate access by enzymes to starch granules, insufficient time for hydrolysis, limited glucose absorption, insufficient intestinal mucosal enzymes, and insufficient pancreatic α-amylase are possible causes.

Recent work has shown that infusing protein with starch into the small intestine enhanced small-intestinal starch disappearance and net portal appearance of glucose over infusing starch alone. In the present experiment, our objective was to evaluate the influence of abomasally infused starch and casein on pancreatic exocrine secretions.

Experimental Procedures

One Holstein, two crossbred, and five Angus steers (305 ± 5 kg) surgically fitted with pancreatic pouch-duodenal re-entrant

cannulas and abomasal infusion catheters were used in a replicated four-by-four Latin square experiment. Steers were housed in individual (2.5-by-2.5 m) pens within a temperature- (23°C) and light- (16 hours light, eight hours dark) controlled room with water available by free choice. The University of Kentucky Institutional Animal Care and Use Committee approved all procedures.

Before surgeries, steers were withheld from feed (48 hours) and water (24 hours). Pancreatic pouch-duodenal re-entrant cannulas and abomasal infusion catheters were placed during an aseptic surgery under general anesthesia. Anesthesia was introduced with 8 mg/kg BW of thiopental sodium and maintained with halothane in oxygen. All animals were allowed to recover a minimum of 14 days before being placed on treatment. Rectal temperatures were checked for seven days after surgery as an indicator of animal health.

The re-entrant cannula linked a pouch of duodenum containing the primary pancreatic duct with the duodenum These cannulas were exteriorized and connected in a manner allowing all pancreatic juice to flow into the duodenum. A 5-cm section of 1.11-cm i.d. and 1.75-cm o.d. silicone tubing was used to connect the cannulas externally.

Abomasal catheters were made from Tygon (0.64-cm i.d. and 0.95-cm o.d.) and placed into the pyloric region of the abomasum, with the remaining tubing exteriorized through the adjacent body wall. Patency was maintained by filling the catheter with mineral oil between infusions.

Steers were weighed at the beginning of each period, and individual DMI adjusted to 1.5 times the NE_m requirement. The diet contained 90% corn silage and 10% supplement on a DM basis (Table 1). Diets were balanced to meet the CP requirements of a 318-kg steer gaining 0.45 kg/day. Each day, silage and supplement were weighed out separately, thoroughly mixed, divided into 12 equal portions, and fed at two-hour increments.

Abomasal infusions consisted of 1,050 g/day (DM basis) of raw corn starch and 0, 60, 120, or 180 g/day (DM basis) sodium casein suspended in tap water to yield a total of 6,000 g/d. They were prepared fresh daily and infused over a 24-hour period using a peristaltic pump. Solution homogeneity was maintained by rapid, continuous mixing with a stir bar and stir plate. Each period began with three days of adaptation by infusing 25%, 50%, and 75% of the final starch and treatment casein levels in 6,000 g/day of total solution. Full treatment levels were infused for eight days, with samples being collected the final day. A minimum of seven days rest was allowed between infusion treatments.

On the sampling day, pancreatic fluid was continuously collected under vacuum into an Erlenmeyer flask in an ice bath for seven hours. At 30-minute intervals, the flasks were switched and incremental sample weights and pH measured. A composite sample was formed for each steer by retaining 10% of the 30-minute samples. Remaining pancreatic fluid was returned to the duodenum using a catheter tip syringe.

Samples were stored (-30°C) until analysis for total protein and activities of α-amylase, trypsin, and chymotrypsin. Enzyme activities were expressed as Units with 1 Unit (U) being equal to the production of 1 µmol of product/minute per volume of pancreatic fluid.

Statistical Analyses

Data were analyzed as a replicated four-by-four Latin square. Linear and quadratic orthogonal contrasts were used to separate treatment means. Results were considered significant at the $P < 0.05$ level.

Results and Discussion

The quantity of pancreatic juice secreted (Table 2) was not altered (average 155 mL/hour; $P > 0.10$) by the infusion of casein with starch. Treatments also had no effect ($P > 0.10$) on the mean pH (8.13) or protein concentration (10.23 mg/ml) of the juice secreted from the pancreas. No differences were detected ($P > 0.10$) in the rate of protein secretion (avg. 1,536 mg/hour).

Casein infusion linearly increased the α-amylase concentration (U/mL; $P < 0.02$) and specific activity (U/mg of protein; $P < 0.03$) of pancreatic α-amylase. These changes corresponded to a linear increase ($P < 0.01$) in the secretion rate of α-amylase activity (U/hour). Infusion of casein with starch did not change ($P > 0.10$) the trypsin or chymotrypsin concentrations (U/L), specific activities (U/mg protein), or secretion rates (U/hour).

The importance of supplying high-quality dietary protein along with dietary carbohydrates on maximizing pancreatic protein secretions has been demonstrated in nonruminants. However, differences between the digestive systems of ruminants and nonruminants likely affect pancreatic protein secretions. In the nonruminant, a postprandial intermittent flow of dietary nutrients enters into the small intestine, resulting in a flux of metabolites and exocrine pancreatic secretions closely related to the nonruminant's feeding pattern and diet. In ruminants, pre-gastric fermentation of substrates in the rumen produces a more continuous flow of nutrients from dietary and microbial origin that are less variable than the diet consumed. This appears to produce a more consistent flow of protein from the pancreas.

Effects reported on pancreatic protein secretion due to the protein flow to the duodenum or the balance of energy and protein flow may also be an important aspect in regulating the enzymatic profile of pancreatic exocrine secretions. As increasing quantities of casein were infused with starch in this study, the concentration, specific activity, and secretion rate of α-amylase increased.

While linear increases were detected for all α-amylase measurements, the 120 g/day treatment concentration, specific activity, and total secretion were lower than 60 g/day treatment. The concentrations and specific activities of α-amylase of the 120 g/day treatment are higher than the control values. However, the 120 g/day treatment had the lowest pancreatic juice secretion rate that resulted in the numerically lowest α-amylase secretion. Individual animal observations (data not shown) confirmed that this tendency was consistent for six of the eight steers.

An estimate of the additional UIP that would be necessary to meet steers' growth potential with infused starch was made. We used the NRC (1996) model, the animal and nutrient parameters from the present study, and a digestibility of 70% for infused carbohydrate to make the estimate. The infusion of

starch increased energy intake from 1.5 x NE_m for the basal diet to 1.84 x NE_m. With this increase in energy intake, estimated daily gain is increased by 290 g/day. The estimated metabolizable protein supply that is necessary to obtain this gain is 85 g/day. Our diet was not designed to supply the additional metabolizable protein. With the postruminal introduction of carbohydrates, no additional bacterial protein production can be assumed. Therefore, additional protein would need to be supplied as UIP or infused postruminally.

Our diet is estimated to supply an adequate quantity of metabolizable protein to meet the energy predicted rate of gain from the basal diet. If the casein infused is assumed to be 100% digestible and the rate of gain estimated from dietary and infused energy were truly obtained, the 180 g/day casein treatment would exceed the metabolizable protein requirement by 47 g/day.

Summary

The objective of the experiment was to evaluate the effect of postruminal protein infusion on pancreatic exocrine secretions. Steers were limit-fed (1.5 x NE_m; 12 equal portions/day) a corn-silage-based diet (90%; DM basis) formulated to contain 12.5% crude protein and were abomasally infused with 1,050 g/day of raw corn starch and 0, 60, 120, or 180 g/day sodium casein suspended in water to yield 6,000 g/day of infusate daily. Periods consisted of three days of adaptation, seven days of full infusion, one day of collection, and seven days of rest. Pancreatic juice was continuously collected for six hours with samples aliquoted at 30-minute intervals. Total juice secretion (155 g/hour; $P > 0.10$) and pH of pancreatic juice (8.13; $P > 0.10$) were not altered by infusion of casein. The secretion rate (1,536 mg/hour; $P > 0.10$) and concentration (10.2 mg/mL; $P > 0.10$) of protein in pancreatic secretions were not affected by infused casein. Casein infusion linearly increased α-amylase concentration (182 to 271 units/mL; $P < 0.02$ and 17.5 to 24.6 units/mg protein; $P < 0.03$) and secretion rate (26,847 to 41,894 units/hour; $P < 0.01$). Infusion did not change ($P > 0.10$) trypsin and chymotrypsin concentrations (1,379 and 349 units/L or 0.134 and 0.033 units/mg protein, respectively) or secretion rates (206 and 52 Units/hour, respectively). Our results indicate that when diets supplying starch to the small intestine are being worked with, the small-intestinal protein supply, in addition to meeting the protein needs of the animal, also appears to be important in maintaining or stimulating α-amylase production. If starch digestion and assimilation in the small intestine are limited by the activity of α-amylase, additional protein could increase the energy available from the diet to the animal.

Table 1. Diet composition for steers infused with casein.

Item	Percentage, DM Basis
Ingredient	
Corn silage	90
Supplement	
Ground corn	2.11
Soybean meal	3.1
Corn gluten meal	2
Molasses	0.5
Urea	0.8
Limestone	1
Trace mineral salt[a]	0.47
Vitamin premix[b]	0.03
Composition	
DM	45.8
CP[c]	12.6
NDF[c]	38.4

[a] 98.5% NaCl, 0.35% Zn, 0.34% Fe, 0.20% Mn, 330 ppm Cu, 70 ppm I, 50 ppm Co, and 90 ppm Se.

[b] 8,800 IU/g vitamin A, 1,760 IU/g vitamin D, and 1.1 IU/g vitamin E.

[c] Values based on analyses of composite samples.

Table 2. Influence of postruminal protein infusion on secretion, pH, protein, α-amylase, trypsin, and chymotrypsin.

	Treatment					P Value	
Observation	**0**	**60**	**120**	**180**	**SEM[a]**	**Linear**	**Quadratic**
Secretion, mL/h	155	167	143	155	11	0.66	0.98
pH of secretion	8.09	8.14	8.16	8.13	0.04	0.51	0.37
Protein							
mg/mL	10.35	9.65	10.03	10.87	0.64	0.51	0.25
mg/h	1,482	1,604	1,414	1,645	117	0.58	0.65
α-Amylase							
Units/mL[b]	182	210	201	271	21	0.02	0.34
Units/mg protein	17.5	21.3	19.8	24.6	1.9	0.03	0.79
Units/h	26,847	36,385	26,763	41,894	2,290	0.01	0.24
Trypsin							
Units/L	1,537	1,252	1,309	1,418	116	0.57	0.11
Units/mg protein	0.143	0.130	0.131	0.133	0.010	0.50	0.44
Units/h	216	210	182	217	19	0.77	0.30
Chymotrypsin							
Units/L	354	296	337	407	42	0.30	0.15
Units/mg protein	0.033	0.030	0.034	0.036	0.003	0.39	0.57
Units/h	50	47	49	63	7	0.26	0.25

[a] SEM = standard error of mean; n = 8.

[b] One unit = 1 µmol product formed per min.

Intestinal Starch Disappearance Increases in Steers Abomasally Infused with Protein

C.J. Richards, A.F. Branco, D.W. Bohnert, D.L. Harmon, and G.B. Huntington

Introduction

Starch that is consumed by ruminants first undergoes ruminal digestion. The extent of ruminal digestion varies and influences the quantity of starch flowing to the small intestine. The amount digested in the rumen is affected by the quantity consumed and the extent of processing. Of the starch consumed, 6% to 60% may escape ruminal digestion and be available for small-intestinal digestion. With normal production intakes of finishing steers, 500 g to 2,000 g of starch may enter the small intestine. Digestion of starch in the small intestine has been calculated to be more energetically efficient than ruminal fermentation. However, shifting digestion from the rumen to the small intestine has not consistently shown increases in feed efficiency. This suggests that ruminants have a limited ability to utilize starch in the small intestine. This study was conducted to evaluate and quantify the relationship between protein supply to the small intestine and extent of starch disappearance in the small intestine.

Experimental Procedures

Animals and Diets

Five Angus steers (345 kg), surgically fitted with ruminal and double-L-shaped duodenal and ileal cannulas, were used in a five-by-five Latin square design experiment. Infusion treatments consisted of raw corn starch (1,050 g) combined with 0, 50, 100, 150, or 200 g of casein and water for a total of 6,000 g/day of solution. Solutions were mixed daily and continuously infused into the abomasum over 24 hours by passing tubing and a retaining washer through the reticular-omasal orifice into the omasum. Steers were housed in individual 2.5-m-by-2.5-m pens in a temperature- (73°F) and light- (16 hours of light) controlled room with water available free choice.

A corn-silage-based diet was formulated to contain 11.7% CP with a supplement (7% DM basis) containing 3.1% soybean meal and 0.5% urea (Table 1). Analysis of nitrogen in composite silage and supplement samples collected over the last four days of each period resulted in an actual average dietary CP content of 11.7%. Dry matter intakes were set at 1.65% BW and divided into 12 equal portions and fed every two hours. Chromic oxide was included at 0.20% of the diet as an indigestible digesta flow marker.

Sample Collection and Analysis

Infusion periods were 14 days, with three days rest between periods. Samples were taken over a 12-hour period on Days 13 and 14. Ileal contents and feces were collected every four hours, while duodenal contents were collected every two hours. Ruminal fluid was collected on Day 13 every four hours for 12 hours, analyzed for pH, deproteinized with meta-phosphoric acid, and frozen. Duodenal, ileal, and fecal samples were immediately frozen and then thawed and composited by animal and period before lyophilizing. Amounts of starch and casein infused were included as intake in the calculations and tables.

Statistics

Data were analyzed as a five-by-five Latin square design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model contained period, steer, and casein infusion treatment. Linear and quadratic orthogonal contrasts were performed for the effects of casein infusion treatment. Treatment effects were considered different when $P < 0.05$. Ruminal VFA, ammonia N, and pH data collected at set times over the sampling period were analyzed as repeated measures. No treatment x time interactions were detected; therefore, measurements were averaged across time, and treatment means compared as previously described. All nutrient disappearance values were calculated as a percent of the nutrient flowing into that segment. Due to failure of ileal cannulas in two steers, we were unable to obtain intestinal digestibility results for two animals on the 100 g casein treatment. The two observations without ileal measures were not included in statistical analysis, and least square means were calculated.

Results and Discussion

Organic Matter Disappearance

Organic matter flow at the duodenum, ileum, and feces was not affected by casein infusion (Table 2). Apparent OM disappearance (quantity or percentage of intake) in the rumen did not differ due to treatment. Duodenal OM flow did show a tendency ($P < 0.09$) to increase linearly with increased casein infusion. The duodenal OM flow for the 200 g/day casein was 475 g/day greater than control (0 casein). The increased duodenal flow was in excess of the 200 g/day difference attributable to casein infusion between the treatments. The additional difference can be accounted for by a tendency ($P < 0.18$) of the stomach OM disappearance to decrease linearly with increased casein infusion. Differences in stomach disappearance may be due to postruminal control of digesta passage. Despite the tendency for differences in duodenal OM flow, ileal OM flows were not different among treatments. This was the result of the quantity (g/day) and percent of apparent OM disappearance (percentage of duodenal flow) in the small intestine increasing linearly ($P < 0.05$) with increased casein infusion. The 200 g/day casein treatment had 602 g/day greater small-intestinal OM digestion than the control treatment. When expressed as a percentage of the duodenal OM flow, small-intestinal OM digestion increased from 42% to 49%. The quantity and percent of apparent OM digestion in the large intestine and total tract were not affected by the infusion of casein.

Protein Disappearance

The quantity and percentage of CP apparently digested in the stomach were not affected by abomasal infusion of casein (Table 3). For all treatments, the corrected CP flow leaving the rumen was greater than CP intake. Ruminal CP outflow, corrected for infused CP, ranged from 109% to 142% of CP in-

Table 1. Diet composition.

Item	%, DM Basis
Corn silage	93.00
Supplement	7.00
Supplement Composition, %	
Ground corn	11.82
Soybean meal	44.41
Urea	7.14
Limestone	12.71
Dicalcium phosphate	7.57
Choice white grease	6.43
Chromic oxide	2.85
Trace mineral salt[a]	6.71
Vitamin premix[b]	0.36
Nutrient Composition	
Dry matter[c]	37.3
Organic matter[c]	93.6
Crude protein[c]	11.7

[a] 98.5% NaCl, 0.35% Zn, 0.34% Fe, 0.20% Mn, 330 ppm Cu, 70 ppm I, 50 ppm Co, and 90 ppm Se.

[b] 8,800 IU/g vitamin A, 1,760 IU/g vitamin D, and 1.1 IU/g vitamin E.

[c] Values based on analyses of composite samples.

Table 2. Apparent organic matter disappearance in steers abomasally infused with starch and protein.

	Casein Infused, g/d						P <	
Item	0	50	100	150	200	SEM[a]	Linear	Quadratic
Intake, g/d	5,824	5,740	5,855	5,739	5,805	56	---	---
OM infusion, g/d	1,032	1,094	1,131	1,189	1,234	9	---	---
Duodenal flow, g/d	4,274	3,914	5,120	4,631	4,749	409	0.09	0.56
Ileal flow, g/d	2,480	2,322	2,761	2,412	2,352	257	0.77	0.45
Fecal flow, g/d	2,216	1,989	2,357	1,907	1,998	256	0.37	0.81
Disappearance, g/d								
Stomach	2,582	2,920	1,866	2,297	2,289	384	0.18	0.50
Small intestine	1,795	1,592	2,359	2,219	2,397	287	0.02	0.87
Large intestine	264	333	404	505	354	90	0.10	0.15
Total tract	4,640	4,845	4,629	5,021	5,041	223	0.07	0.73
Disappearance,[b] %								
Stomach	44.3	50.7	33.5	40.0	39.7	6.2	0.18	0.59
Small intestine	42.0	40.4	44.9	47.7	49.0	3.8	0.03	0.72
Large intestine	10.7	14.4	16.2	20.0	14.5	3.8	0.14	0.17
Total tract	67.7	70.9	66.9	72.4	71.5	3.3	0.23	0.89

[a] SEM = highest standard error of the mean; n = 5 for 0, 50, 150, and 200 g/d of casein infused; n = 3 for 100 g/d of casein infused.

[b] Disappearance as percent of flow to the segment.

takes. Intestinal flows that are greater than intake occur because CP is being recycled to the rumen.

Casein infusion increased (P < 0.01) CP flow at the duodenum. Infused CP for the 200 g/day casein treatment was 173 g/day, while duodenal CP flow for that treatment, including infused CP, was 191 g/day higher than control. Ileal flows of CP were not affected by casein infusion. Therefore, the linear increase (P < 0.01) in quantity and percent (percent duodenal flow) of apparent CP digestion in the small intestine paralleled the infusions of casein and differences in duodenal CP flow. The percent of small-intestinal-apparent CP disappearance, as a percentage of duodenal flow, increased from 63% for control to 70% for 200 g/day casein. The quantities and percentages (percent ileal flow) of large-intestinal-apparent CP disappearance were not different and slightly negative. Negative large-intestinal CP disappearances suggest microbial fermentation was occurring in the large intestine and resulting in microbial CP appearing in the feces. Negative values are commonly reported when digestible carbohydrates flow past the small intestine into the cecum and large intestine. Similar to small-intestinal disappearance, the quantity of apparent total-tract CP disappearance increased (P < 0.01) with increasing casein infusion. However, when expressed as a percent of CP intake, total-tract disappearance showed a tendency (P < 0.06) for a linear increase.

Starch Disappearance

The quantity of apparent starch disappearance in the stomach tended to decrease (Table 4; P < 0.07), and the percent of apparent starch disappearance decreased (P < 0.01) in the stomach with abomasal casein infusion. Starch disappearance in the stomach paralleled the tendency observed for OM disappearance in the stomach. The reduction in stomach starch digestibility resulted in a linear increase (P < 0.01) in duodenal starch

Table 3. Apparent crude protein (CP) disappearance in steers abomasally infused with starch and protein.

	Casein Infused, g/d						P <	
Item	0	50	100	150	200	SEM[a]	Linear	Quadratic
Intake, g/d	732	721	732	718	727	7	---	---
CP infusion, g/d	0	43	86	130	173	1	---	---
Duodenal flow, g/d	919	831	1,129	1,075	1,110	93	0.01	0.71
Ileal flow, g/d	338	296	363	327	335	32	0.72	0.97
Fecal flow, g/d	356	310	372	335	344	40	0.99	0.93
Disappearance, g/d								
Stomach	-188	-67	-311	-227	-210	89	0.31	0.66
Small intestine	582	535	766	748	775	68	0.01	0.63
Large intestine	-19	-14	-9	-8	-9	13	0.41	0.73
Total tract	375	454	446	513	556	36	0.01	0.98
Disappearance,[b] %								
Stomach	-26.1	-9.2	-40.3	-31.7	-29.0	12.0	0.28	0.75
Small intestine	63.2	64.4	67.6	69.6	69.5	1.5	0.01	0.41
Large intestine	-5.5	-5.0	-2.3	-2.5	-2.7	3.8	0.35	0.71
Total tract	51.1	59.5	55.2	60.6	62.0	4.9	0.06	0.76

[a] SEM = highest standard error of the mean; n = 5 for 0, 50, 150, and 200 g/d of casein infused; n = 3 for 100 g/d of casein infused.

[b] Disappearance as percent of flow to the segment.

flow with increased casein infusion. As greater quantities of starch flowed past the duodenum, the percentage of starch digested in the small intestine did not change. This resulted in greater quantities ($P < 0.01$) of starch disappearing in the small intestine. Differences in duodenal starch flow were altered by digestion in the small intestine, which resulted in the quantities of starch flowing past the ileum and appearing in the feces not being affected by treatment.

While starch infusion in the current study is 43 g/hour, duodenal flows range from 53 to 67 g/hour, with an average of 60 g/hour. The average percentage and quantity of starch disappearing in the small intestine for all five of the current treatments is 54% and 34 g/h. Although greater quantities of starch are flowing to the duodenum with increased casein infusion in the current study, there is no change in the disappearance percentage. Also, apparent large-intestinal and total-tract starch disappearances were unaffected by treatment.

In the current study, regressing percent starch disappearance on percent CP disappearance results in a poor relationship ($r^2 = 0.31$). The expression of intestinal digestibility as a percentage of nutrients flowing to the segment does not account for potential differences in the extent of nutrient digestion. This suggests that the relationship between the quantities of N and starch disappearing may be of greater significance. We regressed starch disappearance as a function of the quantity of CP disappearing in the current study: $y = 1.223x - 29.114$ ($r^2 = 0.87$), where x is the quantity of small-intestinal protein disappearance and y is equal to the quantity of starch disappearance. If the regression intercept is set to 0, $y = 1.1812x$ ($r^2 = 0.87$). These regressions indicate that increases in the quantity of CP disappearing are positively associated with increases in the quantity of starch disappearing.

Table 4. Apparent starch disappearance in steers abomasally infused with starch and protein.

	Casein Infused, g/d						P <	
Item	**0**	**50**	**100**	**150**	**200**	**SEM[a]**	**Linear**	**Quadratic**
Intake, g/d	1,557	1,585	1,539	1,590	1,584	40	---	---
Infused, g/d	1,032	1,047	1,038	1,049	1,046	8	---	---
Duodenal flow, g/d	1,303	1,262	1,607	1,444	1,539	98	0.01	0.44
Ileal flow, g/d	559	680	709	619	570	136	0.90	0.28
Fecal flow, g/d	168	245	209	118	149	6	0.22	0.41
Disappearance, g/d								
Stomach	1,286	1,370	970	1,195	1,092	126	0.07	0.52
Small intestine	744	583	897	826	970	84	0.01	0.38
Large intestine	391	434	501	501	423	113	0.61	0.38
Total tract	2,421	2,387	2,368	2,522	2,484	87	0.20	0.53
Disappearance,[b] %								
Stomach	82.8	86.2	61.2	72.8	67.5	6.3	0.01	0.33
Small intestine	56.0	46.5	53.2	57.0	58.8	8.1	0.38	0.43
Large intestine	71.4	64.5	70.4	81.9	75.2	7.3	0.15	0.79
Total tract	93.6	90.5	91.9	95.5	94.4	2.3	0.22	0.38

[a] SEM = highest standard error of the mean; n = 5 for 0, 50, 150, and 200 g/d of casein infused; n = 3 for 100 g/d of casein infused.

[b] Disappearance as percent of flow to the segment.

Plasma and Ruminal Measures

Treatments did not affect plasma glucose or urea concentrations (Table 5). Ruminal pH and concentrations of ammonia were not affected by abomasal infusion of casein. Ruminal pH was not expected to change with abomasal infusion and was monitored to ensure the effectiveness of our abomasal infusions. If starch infused through the reticular-omasal orifice was being digested in the rumen, ruminal pH could have decreased. Ruminal ammonia concentrations ranged from 4.63 mM to 5.58 mM. Total ruminal VFA concentrations increased linearly ($P < 0.02$) with abomasal casein infusion. However, molar percentages of individual ruminal VFA were not affected by casein infusion in the current study. Because all animals were fed equally, differences in total VFA concentrations without changes in molar percentages may be due to a decrease in rumen volume associated with greater flows of duodenal OM as casein infusion increased.

Table 5. Ruminal and blood characteristics in steers abomasally infused with starch and protein.

	Casein Infused, g/d						P <	
Item	**0**	**50**	**100**	**150**	**200**	**SEM[a]**	**Linear**	**Quadratic**
Plasma glucose, mg/dL	61.4	65.6	68.5	62.8	63.5	4	0.86	0.21
Plasma urea, mM	1.71	1.56	1.69	1.62	1.43	0.29	0.45	0.76
Ruminal ammonia N, mM	4.63	5.81	6.02	6.58	5.59	0.97	0.23	0.19
Ruminal pH	6.59	6.58	6.57	6.55	6.58	0.04	0.41	0.51
Ruminal VFA, mol/100 mol								
Acetate	66.8	65.0	66.7	64.5	66.3	0.9	0.49	0.22
Propionate	16.5	17.7	16.5	17.7	16.2	1.0	0.80	0.29
Butyrate	11.7	13.0	11.4	13.3	12.5	0.8	0.24	0.79
Isobutyrate	1.5	1.4	1.5	1.5	1.6	0.1	0.06	0.35
Isovalerate	2.4	1.7	2.6	1.7	2.0	0.3	0.34	0.74
Valerate	1.2	1.3	1.3	1.3	1.3	0.1	0.20	0.39
Total VFA, mM	93.9	95.6	104.2	101.1	101.3	3.3	0.02	0.16

[a] SEM = highest standard error of the mean; n = 5 for 0, 50, 150, and 200 g/d of casein infused; n = 3 for 100 g/d of casein infused.

These results suggest that for diets with large quantities of starch reaching the duodenum, simultaneously supplying protein to the small intestine might increase the quantity of starch disappearing from the small intestine and subsequent digestive efficiency. Whether small-intestinal starch digestive efficiency can be optimized through diet formulation remains to be determined.

Summary

Steers (379 ± 10 kg) with ruminal, duodenal, and ileal cannulas were used in a five-by-five Latin square digestion trial to quantify and evaluate the relationship between intestinal protein supply and intestinal starch disappearance. Treatments were infusion of 0, 50, 100, 150, or 200 g/day of casein along with 1,042 g/day of raw corn starch. Abomasal infusions were accomplished by passing tubing and a pliable retaining washer through the reticular-omasal orifice into the abomasum. Steers were fed at 1.65% BW in 12 equal portions daily. Periods lasted 17 days (12 days of adaptation, two days of collections, and three days of rest). Quantity and percent of OM and protein disappearance from the small intestine increased linearly ($P < 0.03$) with infused casein. Greater quantities of starch disappeared with increased casein infusion ($P < 0.01$). The 200-g/day casein infusion increased small-intestinal starch disappearance 226 g/day over the control. Casein infusion did not affect the quantity or percent of OM, starch, or protein disappearance in the large intestine. Treatments did not change ruminal ammonia N, ruminal pH, or plasma glucose concentrations. Starch disappearance from the small intestine was increased by greater protein flow to the duodenum of steers. These results suggest that for diets with large quantities of starch reaching the duodenum, simultaneously supplying protein to the small intestine might increase the quantity of starch disappearing from the small intestine and subsequent digestive efficiency.

Ruminal versus Abomasal Carbohydrate Infusion Alters Glucose Metabolism in Steers

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Introduction

Starch consumed by ruminants undergoes ruminal digestion before entering the small intestine. The amount of starch digested in the rumen is affected by the quantity consumed and the extent of grain processing. Because of this, diets supply different quantities of starch to the small intestine in ruminants. Of the starch consumed, 18% to 42% may escape ruminal digestion and be available for small-intestinal digestion.

Under normal production, intake of finishing steers would supply 500 to 2,000 g/day of starch to the small intestine. Supplying energy through carbohydrate digestion to glucose in the small intestine is theoretically more energetically efficient than ruminal fermentation of carbohydrates to volatile fatty acids. However, shifting starch digestion from the rumen to the small intestine has not consistently shown increases in feed efficiency. This suggests that site of digestion and the resulting form of energy (volatile fatty acid days glucose) affect efficiency of energy metabolism in ruminants through alteration of energy supplied and/or utilization. Therefore, the objectives of this experiment were to evaluate the effect of site of starch infusion on gastrointestinal energy flux and glucose metabolism in steers.

Procedures

Animals

Eight Angus steers (235 ± 6 kg, average weight) were used in a crossover design. Steers were fitted with permanent ruminal cannulas and abomasal infusion catheters for treatment infusion. Steers were also surgically fitted with chronic indwelling catheters in the hepatic portal vein, hepatic vein, two mesenteric veins, and a mesenteric artery. The right carotid artery was also elevated to provide access to arterial blood in the event a mesenteric arterial catheter became nonpatent. Before surgeries, steers were withheld from feed (48 hours) and water (24 hours). Catheters were placed and the carotid artery was elevated during an aseptic surgery under general anesthesia. All animals were allowed to recover a minimum of 14 days before being placed on treatment. The University of Kentucky Institutional Animal Care and Use Committee approved all procedures. Blood catheters were filled with 1,000 U/ml heparinized saline solution between sampling periods to maintain patency. Patency of the abomasal infusion catheter was maintained by filling with mineral oil between infusions.

Steers were tethered in individual (2.5-by-2.5-m) pens for the first 11 days of infusion and then moved to 2.2-m-by-0.9-m metabolism crates for the final three days of each period. Steers were previously adapted to the metabolism crates to reduce excitement or stress on the day of sample collection. The left jugular vein and elevated right carotid artery were fitted with temporary catheters 12 to 16 hours prior to sampling. Both the pens and metabolism crates were temperature- (23°C) and light- (16 hours light, eight hours dark) controlled rooms, and water was available free choice.

Diets and Infusion Treatments

Steers were weighed at the beginning of each period and individual intake adjusted to 1.5 times the NE_m requirement. A pelleted diet (Table 1) containing 2.41 Mcal/ kg metabolizable energy (ME) was formulated to meet the degradable intake protein requirement of steers receiving ruminal starch infusion and the metabolizable protein requirement of a steer 250 kg gaining 0.84 kg/day (NRC, 1996). The diet was weighed out daily and fed in 12 equal portions at two-hour intervals using automated feeders (Ankom Co., Fairport, N.Y.).

Treatments were ruminal or abomasal infusion with 800 g/day of partially hydrolyzed starch (SH; 16%) for 14 days. The first six days were used for adaptation by infusing 25%, 50%, and 75% of the final SH quantity. Equal quantities of tap water were infused into the site opposite the SH.

Collection

On the last day of infusion, a primed (40 µCi) continuous infusion (0.8 µCi/min) of [U-^{14}C] glucose was infused through a sterile 0.45-µm filter. A primed (15 mL) continuous infusion (0.8 mL/min) of p-amino hippurate (PAH; 10% wt/vol; pH 7.4) was begun into a mesenteric vein through a sterile 0.45-µm filter at 240 minutes.

At 300, 360, 420, 480, 540, and 600 minutes, simultaneous arterial, hepatic, and portal blood samples (15 mL) were taken over a five-minute period. Samples were collected into heparinized syringes, transferred to centrifuge tubes containing 2 mg NaF per mL of blood, mixed by inversion, and placed on ice until centrifugation (15,000 x g; 15 minutes at 4°C), and plasma harvested. At each sampling time, an additional 2 mL of arterial, hepatic, and portal blood were collected into 5-mL heparinized glass syringes, capped, and immediately analyzed for hemoglobin and O_2 saturation using a Hemoximeter (Radiometer America, Westlake, Ohio).

Analyses

All samples were analyzed for glucose-specific activity by simultaneously passing through a cation (AG 50W; BioRad Laboratories, Hercules, Calif.) and anion (BIO-REX 5; BioRad Laboratories, Hercules, Calif.) exchange column. The columns were rinsed by allowing 5 mL of H_2O to completely pass through both columns. The total eluent was collected, lyophilized, and reconstituted in 1 mL of H_2O. An aliquot (700 µL) was combined with 15 mL of scintillation cocktail and counted on a liquid scintillation counter. The remaining sample was analyzed for glucose concentration using glucose dehydrogenase.

Hourly arterial, hepatic, and portal plasma samples were also analyzed for glucose, lactate, PAH, ammonia N, β-hydroxybutyrate, α-amino N, and VFA. Glucose and lactate plasma concentrations were determined with a YSI Model 27 analyzer (Yellow Springs Instruments, Yellow Springs, Ohio).

Table 1. Diet composition.

Item	%, DM Basis
Intake, kg dry matter	4.49
Ingredients	
Orchardgrass hay	89.45
Corn gluten meal	5.00
SoyPass Protein™,[a]	5.00
Mineral mix[b]	0.50
Vitamins[c]	0.05
Nutrient Composition	
DM, %[d]	89.2
Crude protein, %[d]	19.8
NDF, %[d]	66.5
ME, Mcal/kg[e]	2.41

a Soybean meal treated with lignosulfonate (Ligno-Tech USA, Fort Wayne, IN).
b 98.5% NaCl, 0.35% Zn, 0.34% Fe, 0.20% Mn, 330 ppm Cu, 70 ppm I, 50 ppm Co, and 90 ppm Se.
c 8,800 IU/g vitamin A, 1,760 IU/g vitamin D, and 1.1 IU/g vitamin E.
d Values based on analyses of composite samples.
e Calculated from NRC 1996 values.

Plasma was analyzed for PAH (colorimetrically) and ammonia N (glutamate dehydrogenase). The PAH standards were prepared from the infusion solutions. Plasma was deproteinized with an equal volume of 0.6 N $HClO_4$, centrifuged (15,000 x g 10 minutes), and the supernatant analyzed for α-amino N (colorimetric) and β-hydroxybutyrate (Sigma Diagnostics procedure 310-UV). Two mL of each hourly sample were used to form a single composite for determination of VFA content by gas chromatography.

Calculations

Net portal-drained visceral (PDV) flux of metabolites was calculated as the product of portal blood flow and portal-arterial concentration difference, net total splanchnic metabolite flux as the product of hepatic blood flow and hepatic-arterial concentration difference, and net hepatic flux as the difference between net total splanchnic flux and net PDV flux. A positive net flux indicates release or absorption of a metabolite, whereas a negative net flux indicates uptake or utilization. Distribution of energy flux was determined from nutrient fluxes and the nutrient heat of combustion. Packed cell volumes were used to calculate blood flows for determination of oxygen and hemoglobin fluxes rather than plasma flows since both analyses were performed on whole blood. A positive net flux indicates a release or net absorption of the nutrient into the plasma pool, whereas a negative net flux implies net uptake or utilization by gastrointestinal tissues. [U-^{14}C] glucose data for the specific activity of plasma [U-^{14}C] glucose was plotted versus time, and the plateau-specific activity was identified and calculated for each infusion by averaging the hourly samples between 300 and 600 minutes of infusion.

Statistics

Data were analyzed as a crossover design with steer, period, and treatment included in the model. Only seven observations were used for nutrient flux and [U-^{14}C] glucose measurements of the ruminal treatment due to catheter function failing in one steer. Results were considered significant if probability of Type 1 error was equal to or less than 0.05.

Results and Discussion

The arterial plasma concentration of glucose was increased (Table 2; P = 0.02) while concentrations of β-hydroxybutyrate, total VFA, acetate, and butyrate were decreased (P = 0.01, 0.01, and 0.05, respectively) in steers receiving abomasal SH. Arterial concentrations of lactate, propionate, α-amino N, ammonia N, and oxygen were not affected by treatment.

The portal-arterial concentration difference of glucose (Table 2; P = 0.01) and 2-methyl butyrate (P = 0.05) were greater, while butyrate (P = 0.01) and valerate (P = 0.01) differences decreased. Portal-arterial concentration differences of oxygen are less negative (P = 0.05) with abomasal SH, and the other metabolites were not changed. Abomasal SH infusion increased the hepatic-portal concentration difference (more negative) of 2-methylbutyrate (P = 0.03) and decreased (less positive or negative) lactate, β-hydroxybutyrate, butyrate, valerate, and

Table 2. Arterial concentrations and venoarterial differences of metabolites.

Item	Treatment		SEM[a]	P <
	Ruminal	Abomasal		
Arterial Concentration, mM				
Glucose	4.59	4.87	0.06	0.02
Lactate	0.51	0.51	0.02	0.84
β-hydroxybutyrate	0.41	0.16	0.03	0.01
Total VFA	1.20	1.00	0.03	0.01
Acetate	1.16	0.97	0.024	0.01
Propionate	0.021	0.021	0.001	0.92
Isobutyrate	ND[b]	ND	---	---
Butyrate	.014	0.009	0.002	0.05
2-methylbutyrate	ND	ND	---	---
3-methylbutyrate	ND	ND	---	---
Valerate	ND	ND	---	---
α-amino N	3.15	2.85	0.10	0.07
Ammonia N	0.165	0.168	0.004	0.58
Oxygen	5.86	5.80	0.09	0.62
Portal-Arterial Difference, mM				
Glucose	-0.06	0.14	0.01	0.01
Lactate	0.102	0.086	0.007	0.17
β-hydroxybutyrate	0.14	0.12	0.01	0.27
Total VFA	1.32	1.31	0.67	0.92
Acetate	0.90	0.96	0.06	0.45
Propionate	0.28	0.25	0.01	0.24
Isobutyrate	0.014	0.016	0.001	0.13
Butyrate	0.103	0.060	0.007	0.01
2-methylbutyrate	0.009	0.011	0.001	0.03
3-methylbutyrate	0.008	0.009	0.001	0.23
Valerate	0.018	0.01	0.001	0.01
α-amino N	0.32	0.22	0.04	0.08
Ammonia N	0.17	0.20	0.02	0.17
Oxygen	-1.69	-1.61	0.02	0.05
Hepatic-Portal Difference, mM				
Glucose	0.25	0.21	0.02	0.14
Lactate	-0.164	-0.084	0.012	0.01
β-hydroxybutyrate	0.10	0.05	0.01	0.01
Total VFA	0.88	1.02	0.04	0.05
Acetate	-0.045	0.016	0.069	0.54
Propionate	-0.261	-0.224	0.015	0.13
Isobutyrate	-0.014	-0.014	0.001	0.51
Butyrate	-0.086	-0.045	0.006	0.01
2-methylbutyrate	-0.009	-0.011	0.001	0.03
3-methylbutyrate	-0.006	-0.007	0.001	0.39
Valerate	-0.018	-0.010	0.001	0.01
α-amino N	-0.18	-0.11	0.04	0.29
Ammonia N	-0.182	-0.217	0.015	0.13
Oxygen	-1.14	-0.90	0.04	0.01

[a] SEM = highest standard error of the mean; n = 7 for ruminal treatment; n = 8 for abomasal treatment.

[b] ND = nondetectable.

Table 3. Portal-drained visceral, hepatic, and total splanchnic flux of metabolites.

Item	Treatment		SEM[a]	P <
	Ruminal	Abomasal		
Portal-Drained Visceral Flux, mmol/h				
Glucose	-30	64	5	0.01
Lactate	41	39	3	0.56
β-hydroxybutyrate	58	56	5	0.72
Total VFA	544	601	34	0.27
Acetate	367	437	27	0.11
Propionate	114	116	8	0.83
Isobutyrate	5.8	7.2	0.4	0.04
Butyrate	42.6	27.4	3.2	0.02
2-methylbutyrate	3.8	4.8	0.1	0.01
3-methylbutyrate	3.1	3.9	0.3	0.08
Valerate	7.5	4.4	0.4	0.01
α-amino N	131	96	18	0.21
Ammonia N	70	91	7	0.07
Oxygen	-945	-999	25	0.16
Hepatic Flux, mmol/h				
Glucose	124	137	9	0.34
Lactate	-75	-39	8	0.02
β-hydroxybutyrate	68	41	5	0.01
Total VFA	-87	-23	44	0.32
Acetate	72	113	37	0.44
Propionate	-106	-99	9	0.07
Isobutyrate	-5.6	-6.2	0.4	0.34
Butyrate	-33.9	-18.6	3.0	0.01
2-methylbutyrate	-3.8	-4.81	0.1	0.01
3-methylbutyrate	-2.4	-2.7	0.2	0.22
Valerate	-7.5	-4.4	0.4	0.01
α-amino N	-58	-33	16	0.01
Ammonia N	-76	-100	7	0.04
Oxygen	-1,066	-943	38	0.06
Total Splanchnic Flux, mmol/h				
Glucose	95	201	11	0.01
Lactate	-33	0.0	11	0.07
β-hydroxybutyrate	126	97	8	0.04
Total VFA	456	577	25	0.02
Acetate	439	549	23	0.02
Propionate	7.8	17.0	2.8	0.06
Isobutyrate	0.2	1.1	0.4	0.12
Butyrate	8.7	8.8	1.0	0.96
2-methylbutyrate	0	0	0	1.0
3-methylbutyrate	0.7	1.2	0.2	0.17
Valerate	0	0	0	1.0
α-amino N	73	63	14	0.63
Ammonia N	-6	-8	1	0.28
Oxygen	-2,010	-1,942	46	0.31

[a] SEM = highest standard error of the mean; n = 7 for ruminal treatment; n = 8 for abomasal treatment.

oxygen (P = 0.01). Starch hydrolysate infusion into the abomasum did not affect other hepatic-portal measures.

Net portal flux of glucose, isobutyrate, and 2-methylbutyrate was greater (Table 3; P < 0.05) with postruminal SH, while butyrate and valerate flux decreased. No differences were detected in the other measures. Hepatic flux of lactate, butyrate, valerate, and α-amino N were less negative (P < 0.05) with abomasal SH, while β-hydroxybutyrate flux decreased (P = 0.01) and ammonia N flux was more negative (P = 0.04). Abomasal SH infusion increased total splanchnic (TS) flux of glucose (P = 0.01), total VFA (P = 0.02), and acetate (P = 0.02) but decreased TS β-hydroxybutyrate flux (P = 0.04). Other nutrient TS flux did not differ.

[U-^{14}C] glucose irreversible loss, PDV glucose utilization (P = 0.03), and PDV glucose production (Table 4) were greater (P = 0.01) when SH was supplied to the small intestine. Hepatic utilization and hepatic production of glucose did not change due to treatment.

Three of the eight steers gave inconsistent portal plasma flows, probably the result of poor mixing of PAH in the portal system; however, the hepatic plasma flows were similar to the other five steers. The average portal plasma flow as a percentage of hepatic plasma flow for the remaining animal observations was 79.8% and not different due to treatment (Table 5). Therefore, these three portal plasma blood flows were calculated as 80% of hepatic flow. Arterial plasma flow was not different, while portal (P = 0.01) and hepatic (P = 0.03) plasma flows were greater when SH infusion was shifted to the abomasum.

Portal-drained visceral energy flux from glucose (P = 0.01), isobutyrate (P = 0.04), and 2-methylbutyrate (P = 0.01) were greater for the abomasal infusion treatment, while butyrate (P = 0.02) and valerate (P = 0.01) were greater with the ruminal infusion (Table 5). The total portal energy flux and other measurements were not different. Total splanchnic energy flux from glucose, acetate, total energy flux, and energy release were greater in steers receiving the abomasal infusion of SH (P = 0.01; 0.02:0.01 and 0.01, respectively). Splanchnic energy flux from β-hydroxybutyrate was greater (P = 0.04) for the ruminal treatment, while α-amino nitrogen (AAN) and oxygen energy were not different.

Table 4. Glucose kinetics using a primed continuous infusion of [U-^{14}C] glucose.

	Treatment			
Item	**Ruminal**	**Abomasal**	**SEM[a]**	**P <**
[U-^{14}C] Glucose Measures, mmol/h				
Irreversible loss	121 (7)	192 (8)	14	0.01
Portal-drained visceral utilization	28 (7)	65 (8)	9	0.03
Portal-drained visceral production	-2 (7)	129 (8)	9	0.01
Hepatic utilization	21 (7)	-14 (8)	18	0.20
Hepatic production	145 (7)	123 (8)	11	0.19

[a] SEM = highest standard error of the mean; n = number in parentheses.

Table 5. Distribution of portal-drained visceral and total splanchnic energy flux.

	Treatment			
Item	**Ruminal**	**Abomasal**	**SEM[a]**	**P <**
Plasma Flow, L/h				
Arterial	109	114	7.9	0.67
Portal	406	455	7.4	0.01
Hepatic	515	568	12.9	0.03
Portal-Drained Energy Flux,[b] kcal/d (Percentage of Total)				
Glucose[c]	0 (0)	1,025 (10.6)	80	0.01
Lactate	324 (3.7)	305 (3.2)	23	0.56
β-hydroxybutyrate	772 (8.8)	737 (7.6)	69	0.72
Acetate	1,845 (21.0)	2,195 (22.8)	136	0.11
Propionate	995 (11.3)	1,015 (10.4)	67	0.83
Isobutyrate	76 (.9)	95 (1.0)	5	0.04
Butyrate	565 (6.4)	362 (3.7)	43	0.02
2-methylbutyrate	61 (.7)	78 (.8)	2	0.01
3-methylbutyrate	50 (.6)	63 (.6)	4	0.08
Valerate	121 (1.4)	72 (.7)	7	0.01
Total VFA	3,715	3,882	214	0.58
α-amino N	1,500 (17.0)	1,099 (11.4)	209	0.21
Oxygen	-2,484 (28.2)	-2,626 (27.2)	65	0.16
Total, kcal/d	8,743	9,676	455	0.18
Portal-drained energy/ME intake, %	64.6	69.1	3.1	0.32
Total Splanchnic Energy Flux, kcal/d (Percentage of Total)				
Glucose[b]	1,524 (13.2)	3,226 (24.6)	172	0.01
β-hydroxybutyrate	1,669 (14.5)	1,283 (9.8)	108	0.04
Acetate	2,205 (19.1)	2,761 (21.1)	116	0.02
α-amino N	836 (7.3)	723 (5.5)	167	0.63
Oxygen	-5,287 (45.9)	-5,107 (39.0)	120	0.31
Total, kcal/d	11,522	13,101	256	0.01
Total splanchnic energy release, kcal/d	6,234	7,993	242	0.01

[a] SEM = highest standard error of the mean; n = 7 for ruminal treatment; n = 8 for abomasal treatment.

[b] Values are calculated from portal-drained visceral flux values using heats of combustion (kcal/mol); glucose, 669.9; lactate, 326.8; α-amino N, 478; β-hydroxybutyrate, 552; acetate, 209.4; propionate, 365; 4-carbon VFA, 552; 5-carbon VFA, 678; and oxygen 4.8934 kcal/liter.

[c] Negative value regarded as zero.

Glucose

Portal Glucose Flux. Increasing postruminal starch consistently results in increased PDV flux of glucose in growing and lactating ruminants. If the flux is calculated as an increase over the ruminal infusion, the portal flux of glucose is 94 mmol/h or 51% of the infused starch.

Hepatic Glucose Flux. It is unclear what effect portal glucose concentrations have on hepatic gluconeogenesis. In fed ruminants and nonlactating cows, exogenous glucose decreases hepatic glucose production by 25%. In lactating and fasted animals, the decrease is as great as 80%. It has been shown in steers that insulin release stimulated by glucose inhibited gluconeogenesis in response to increased exogenous glucose.

The combination of a greater PDV flux and no change in hepatic flux of glucose results in a greater TS flux of glucose for the abomasal treatment. The difference of 106 mmol/hour of TS glucose flux calculates to 458 g/day greater glucose flux. The TS flux relates to the amount of a metabolite that is released into the circulation and is available as glucose supply for use by the peripheral tissues.

Isotope Measures

Irreversible Loss. Irreversible loss of glucose is the glucose that leaves the sampled pool and never returns, thus representing the net quantity of glucose that must be synthesized and/or absorbed from the gut per unit time. Infusion of SH into the abomasum guarantees a supply of readily available carbohydrate to the small intestine, while the ruminal infusion allows equal energy intake comparisons between treatments. Shifting carbohydrate digestion to the small intestine increased irreversible loss by 59%, resulting in greater glucose availability to peripheral tissues.

PDV Utilization and Production

PDV utilization measures the PDV use of glucose that is supplied in the arterial circulation but does not account for any luminal glucose utilized by the intestinal epithelium before it enters circulation. These data show that when starch is digested in the small intestine, glucose use by gut tissues increases. This could simply be the result of the increased glucose availability to gut tissues. Glucose PDV production is the sum of net portal flux and PDV utilization or, stated another way, it is the amount leaving the gut plus the amount that is metabolized. The ruminal infusion treatment had a negative net flux of glucose; however, when SH was infused abomasally, the glucose production increased to 129 mmol/hour, which was equivalent to the amount produced in the liver. This indicates that intestinal digestion can have a profound impact on the glucose economy of the animal.

Other Fluxes

VFA Flux. Supplying additional energy to the rumen through infusion of SH would be expected to increase the ruminal production of VFA. However, no change occurred in total PDV VFA flux. There was an increased PDV flux of isobutyrate and 2-methylbutyrate and a decrease in butyrate and valerate flux when starch was infused into the abomasum. The decrease of approximately 15 mmol/h of butyrate was the only major change in hepatic VFA metabolism due to postruminal starch digestion. The hepatic tissues used numerically less total VFA, resulting in a greater TS flux of total VFA for the abomasal treatment. These differences were unexpected. They may result from negative associative effect of the ruminally infused carbohydrate, resulting in lower ruminal VFA production.

Nitrogen Flux

Increasing the energy supply to the rumen numerically increased PDV AAN flux and decreased ammonia N flux. This would be consistent with an increase in microbial protein production or a greater efficiency of dietary protein use in the rumen as there was no difference in dietary protein between treatments. Ammonia N flux was reduced from 24% to 19% of N intake, while AAN was increased from 26% to 36% of N intake when SH was supplied to the rumen. Hepatic utilization of AAN is 25 mmol/h greater with ruminal starch infusion. This results in the hepatic tissues using 44% and 34% of the PDV AAN flux in the ruminal and postruminal treatments, respectively. Increases in absorbed AAN were utilized by the hepatic tissues, resulting in the TS release of AAN not being affected by treatment. The hepatic tissues in both treatments cleared all portal ammonia N.

Energy Flux

PDV Energy. The total daily energy accounted for as portal flux averaged 9,209 kcal/day, which is 68% of the calculated ME or 55% of the digestible energy (DE) consumed from the pelleted diet and infusion. While the total portal energy fluxes were not different in this study, the abomasal treatment had a net energy flux from glucose, accounting for 10.6% of the total energy, while the ruminal infusion supplied no energy in the form of glucose. However, the ruminal treatment had 16.7 kcal/hour more energy flux from AAN. The average portal VFA energy flux accounts for 28% of the ME energy intake from the diet and SH. Volatile fatty acids accounted for 42.7% and 40.1% of the total portal energy flux for the ruminal and abomasal treatments, respectively. Oxygen consumption averaged 27.6% of the portal energy flux. Heat of fermentation, ketones other than β-hydroxybutyrate, and non-VFA lipids are all components of ME that are not evaluated by these methods.

Despite differences in substrates supplied to the liver, energy consumption did not differ between treatments. Hepatic O_2 usage was 113% and 94% of PDV O_2 usage for the ruminal and abomasal infusions, respectively. The liver released similar quantities of glucose and acetate, and the ruminal treatment released more β-hydroxybutyrate. Percentage of PDV VFA flux used by the liver ranged from 68% to 97%, while the AAN was 44% for the ruminal and 34% for the abomasal treatment. All the PDV flux of ammonia N, 2-methylbutyrate, and valerate were utilized in the liver.

TS Energy Flux. As indicated by oxygen use, there are minimal differences (5.7% increase with abomasal) in energy usage in the PDV and only a numerical difference (13% greater for ruminal infusion) in hepatic tissues. This resulted in similar quantities of TS energy consumption. When expressed as a percentage of TS energy flux, ruminal infusion utilized 46% of the TS energy flux, while the abomasal treatment only consumed 39%.

This would imply an overall greater energetic efficiency for the abomasal treatment; however, these differences in the percentage of energy consumed in the TS tissues are largely a result of lower TS energy flux for the ruminal infusion. Release of energy substrates from the TS tissues accounts for 38% and 48% of the DE supplied to the steers. Greater quantities of glucose and acetate are available to peripheral tissues with abomasal SH, while β-hydroxybutyrate and α-amino nitrogen quantities are greater with ruminal infusion. Further increases in postruminal energy efficiency would have to result from the efficiency with which substrates available to the periphery are utilized.

Summary

Eight beef steers (235 ± 6 kg) were used in a crossover design to quantify interorgan and whole-body glucose metabolism in response to changing site of starch infusion. Steers with permanent ruminal cannulas, abomasal infusion catheters, mesenteric arterial, mesenteric venous, portal venous, hepatic venous, and a raised carotid artery were infused ruminally or abomasally with 800 g/day of partially hydrolyzed starch (SH; 160 g/L) for 14 days. The first six days were used for adaptation by infusing 25%, 50%, and 75% of the final SH quantity. Equal quantities of tap water were infused opposite the SH. A pelleted diet containing orchardgrass (89%), corn gluten meal (5%), and SoyPass (5%) was fed at 1.5 times the NE_m requirement in 12 equal portions daily. A primed continuous 10-hour jugular infusion of [U-^{14}C] glucose and a seven-hour primed continuous mesenteric infusion of p-amino hippuric acid were performed on Day 14. Hourly sets of arterial, portal venous, and hepatic venous blood samples were taken the final six hours.

Shifting the site of starch digestion from the rumen to the small intestine increased ($P < 0.05$) glucose utilization by the PDV tissues, PDV glucose flux, and irreversible loss of glucose. Hepatic glucose production and TS oxygen utilization were not affected. Abomasal infusion resulted in greater ($P < 0.05$) total energy, glucose energy, and acetate energy flux from the TS tissues. These results indicate that shifting starch digestion to the intestine increases PDV glucose uptake and utilization without a decrease in hepatic glucose production, resulting in a greater irreversible loss of glucose.

Intestinal digestion of starch resulted in a greater availability of glucose and acetate to the peripheral tissues. While the gastrointestinal tract utilizes a major portion of the energy consumed, site of digestion did not affect the quantity of energy used by these tissues. Differences in animal performance should, therefore, depend on the efficiency with which peripheral tissues utilize the energy substrates supplied.

Differential Expression of EAAC1 versus GLT-1 Glutamate Transporters Parallels Tissue Concentrations of L- versus D-Glutamate in Growing versus Non-Growing Lambs[a]

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Introduction

The uptake of digesta-derived glutamate and aspartate by the mammalian small-intestinal epithelia results in little net transepithelial flux into portal blood. Thus, the function of anionic transporters that mediate glutamate and aspartate uptake from digesta directly support epithelial functions. In sheep, glutamate is considered a very important substrate for small-intestinal mucosa, as only about 4% of gut-infused [^{14}C] glutamate appears in the portal blood as [^{14}C]-labeled glutamate and/or glutamine. In addition, about 65% to 75% of enterally derived glutamate is oxidized to CO_2 in a single pass. Glutamate is also an important source of metabolic energy for the ovine placenta, wherein more than 90% of fetal circulating glutamate is extracted in a single pass and predominantly oxidized to CO_2. Overall, in forage-fed lactating dairy cattle, about 43% of glutamate is oxidized to CO_2, slightly greater than observed for aspartate, alanine, and glutamine (about 28% to 35%). These extensive oxidation values approximate those observed for acetate, propionate, and butyrate, which are considered the principal sources of metabolic energy in ruminants.

In addition to the importance of glutamate as an oxidizable metabolic fuel for ruminants, glutamate also constitutes a significant (4% to 6.2%), if relatively small, contribution to total plasma glucose produced in sheep. Overall, gluconeogenesis accounts for about 25% of total glutamate turnover in sheep. In terms of reserve gluconeogenic capacity, renal glutamate-derived gluconeogenesis is important, accounting for 33% to 50% of glutamate-derived glucose in fasted and starved sheep.

The absorption and metabolism of D-glutamate by mammals has largely been ignored or considered unimportant, relative to that of L-glutamate. However, the absorption of D-isomers of amino acids by mammalian small-intestinal epithelia does occur. Given that microbial protein typically accounts for the majority of protein digested by ruminants, it is likely that ruminants are exposed to metabolically significant levels of D-glutamate. For example, duodenal digesta collected from cattle fed a 40% grass-silage diet contained 1.72 g of D-glutamate per kg of DM, representing about 4.6% of total glutamate entering the small intestine. In addition, cattle apparently possess a large capacity to metabolize D-glutamate. D-aspartate oxidase (an enzyme that racemizes D-glutamate to alpha-ketoglutarate, which also is the initial metabolite of L-glutamate catabolism) is expressed in peroxisomes by bovine proximal nephron tubules and hepatocytes. In addition, beef kidney displays a 95% substrate specificity for D-glutamate and a K_m of 9.7 mmol/L.

Given the importance of glutamate and aspartate metabolism to tissue-specific and whole-animal amino acid metabolism, knowledge regarding the relationship between glutamate transport proteins and tissue concentrations of glutamate is desirable. Recent research from this lab has determined that of the four non-retinal mammalian, high-affinity, concentrative glutamate transporters, only GLT-1 and EAAC1 are expressed by intestinal epithelial, liver, or kidney tissues of young sheep or adult cattle. With regard to substrate regulation of EAAC1 and GLT-1, evidence exists that the content of GLT-1 and EAAC1 in brain tissue is differentially regulated by L-glutamate. In cell culture models, EAAC1 and/or GLT-1 expression by epithelial cells is sensitive to extracellular L-glutamate concentrations.

In contrast to these *in vitro* models, little is known about regulation of System X^-_{AG} transporters in vivo and about the role that glutamate transport capacity has in support of animal growth. In a gene knockout study, intestinal and kidney phenotypical development of *eaac-1*$^{-/-}$ mice is normal, as are concentrations of amino acids in plasma. However, the concentration of glutamate and aspartate in the urine of these animals is elevated 1,400- and 10-fold, respectively, as compared to wild-type mice. As EAAC1 is localized to the apical (lumen-facing) membrane of renal tubule epithelia, these data indicate the importance of EAAC1 in resorbing glutamate and aspartate from filtered blood.

[a] Abbreviations used: ADF, acid detergent fiber; BW, body weight; CP, crude protein; DM, dry matter; M_r, apparent migration weight; NDF, neutral detergent fiber; OM, organic matter; NE_m, net energy of maintenance; SCFA, short-chained fatty acids.

Given the above information, the objectives of this study were to determine:

- whether the ileal epithelial, hepatic, and/or renal tissue expression of GLT-1 and EAAC1 differed in wethers fed enough of forage-based diets to gain versus maintain body weight (BW), and
- whether any relationship existed between EAAC1 and GLT-1 content and concentrations of D- or L-glutamate.

Procedures

Care of Animals and Tissue Collection

Eighteen cross-bred wether lambs (mean BW = 30 ± 1.2 kg) were randomly assigned to one of two dietary treatments. The lambs were housed and group-fed within dietary treatments in 2.4-square-meter pens for at least 14 days and then placed in metabolism crates for at least 14 days in an environmentally controlled room (21°C) with 16 hours light, eight hours dark photo cycles. Dietary treatments consisted of forage-based diets (Table 1) to meet 1.2 (n = 9) or 2.0 (n = 9) times their NE_m requirements (NRC, 1985) and were fed for at least 14 days. Diets were formulated so that all lambs would receive the same amount of metabolizable protein per day using calculations described by the NRC (1996) for beef cattle.

Sheep were weighed twice a week and the amount of diet fed adjusted accordingly. The average daily gain achieved was calculated over 10 of 14 days that sheep were housed in metabolism crates. Feed and fecal samples were collected the final five days, composited within animal across sampling day, and stored at -30°C. Blood samples were collected by jugular venipuncture three hours after the morning feeding on the day prior to tissue collection, and plasma was harvested after centrifugation (12,000 g for 20 minutes) and stored at -30°C. At the conclusion of the feeding period, animals were anesthetized by intrajugular administration of pentobarbital (80 mg/kg BW), and rumen, small intestine, liver, and kidney removed.

Samples of whole ruminal contents from the midventral region of the rumen were taken, strained through four layers of cheesecloth, and pH of the liquor determined. Five mL of rumen liquor then were acidified with 1 mL of 1.1 mol/L metaphosphoric acid and stored at -30°C. Representative samples (1 g) of liver (caudal lobe), kidney (cortex and medulla), and ileal epithelium were homogenized in 5% trichloroacetic acid and stored at -20°C until analyzed for amino acid content.

Representative samples (2 g) of liver (caudal process) and kidney (cortex and medulla) were homogenized for isolation of cellular membranes. After rinsing in 0.9% NaCl at 4°C, ileal (middle of the distal region) epithelia were scraped (Matthews et al., 1995) and homogenized. RNA was isolated from representative samples (4 g) of liver (caudal lobe), kidney (cortex and medulla), and scraped ileal epithelial tissue.

Analysis of Diet Digestibility, Rumen Fermentation, and Blood Metabolites

Diet and fecal samples were analyzed for dry matter (DM), organic matter (OM), percent crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and gross energy, whereas rumen liquor samples were analyzed for SCFA and plasma samples for SCFA and glucose. Glutamine, alanine, and L- and D-glutamate concentrations in plasma and tissue were determined by combined HPLC and enzymatic-fluorometric analysis.

Table 1. Dry matter intake, components, and composition of diets fed to lambs.[1, 2]

	Dietary Treatment	
Item	**1.2 x NE_m**	**2.0 x NE_m**
DM intake (g/100g body weight)	1.98	3.33
DM intake (g/day)	593	1000
Ingredient	**g/kg DM**	
Orchardgrass hay	700.0	716.6
Solka floc	12.0	154.0
SoyPass	93.6	10.0
Blood meal	74.0	12.0
Urea	4.0	5.0
Molasses	30.0	30.0
Corn oil	50.0	50.0
Ammonium chloride	5.0	5.0
Limestone	16.0	9.0
Dicalcium phosphate	10.0	3.0
Trace mineralized salt[3]	5.0	5.0
Vitamin A, D, and E premix[4]	0.4	0.4
Analysis		
Crude protein (g/kg)	261.1	176.1
NDF (g/kg)	515.1	619.3
ADF (g/kg)	214.7	326.7
Ash (g/kg)	163.3	113.4
Gross energy (kcal/g)	4.64	4.58
Digestible energy (kcal/g)	4.08	4.16

1 Diets were formulated so that lambs would receive similar amounts of metabolizable protein per day. SoyPass and blood meal were used as sources of ruminally undergraded intake protein to balance for metabolizable protein intake.

2 Abbreviations: NE_m = net energy maintenance; DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber.

3 98.5% NaC1, 0.35% Zn, 0.34% Fe, 0.20% Mn, 330 ppm Cu, 70 ppm I, 50 ppm Co, and 90 ppm Se.

4 8800 IU/g vitamin A, 1,760 IU/g vitamin D, and 1.1 IU/g vitamin E.

Immunoblot and Northern Analyses

Tissue homogenates were centrifuged for two minutes at 400 g to remove cellular debris. A plasma-membrane-enriched fraction of cellular proteins was generated by centrifugation of the resulting supernatant at 100,000 g for 30 minutes. The membrane pellet was resuspended, proteins separated by polyacrylamide gel electrophoresis and electrotransferred to a nitrocellulose membrane. GLT-1 and EAAC1 protein expression was evaluated by immunoblot analyses, as previously described by this laboratory. After exposure to autoradiographic film, a digital image of the radiographic bands was recorded and quantified. Apparent migration weights (M_r) were calculated by regression of the distance migrated against the M_r of a 10-to-200 kDa standard.

Total RNA was obtained by an acidic phenol-chloroform extraction and 15 µg of total RNA were size-separated in a 1% agarose gel containing 0.02 M formaldehyde, transferred to a nylon membrane, and cross-linked by UV light. Expression of GLT-1 and EAAC1 mRNA were evaluated using [^{32}P]-labeled

rat cDNA probes specific for GLT-1 and EAAC1. Capture and reproduction of hybridization product images was as described above for immunoblots. Blots were stripped and re-probed (hybridization/wash, 60°/64°C) for 18S rRNA using a [^{32}P]-labeled mouse 18S cDNA probe. Hybridization bands were visualized and quantified by autoradiography as described above. Densitometric data for GLT-1 and EAAC1 mRNA then were corrected for unequal loading (less than 15%) by normalization to relative 18S rRNA mRNA expression.

Statistical Analysis

Data were analyzed as a completely randomized design using the GLM procedures of SAS (SAS 1988).

Results and Discussion

Demonstration of Differential Animal Growth, Digestibility of Diets, and Rumen Liquor SCFA Concentrations

Final BW of the sheep fed the 2.0 x NE_m diet was 4 ± 1.4 kg greater (P = 0.001) than lambs fed the 1.2 x NE_M diet (Table 2). Final BW of lambs fed the 1.2 x NE_m diet did not differ (P = 0.139) from initial BW, whereas lambs fed the 2.0 x NE_m diet gained (P = 0.001) 0.26 ± 0.05 kg/day. Predictably, DM, OM, CP, and energy digestibilities of the 1.2 x NE_mdiet were 1.3% (P = 0.010), 1.6% (P = 0.002), 2.8% (P = 0.001), and 1.6% (P = 0.004) greater than that of the 2.0 x NE_m diet, respectively, whereas NDF digestibility tended (0.08%; P = 0.065) to be greater. In contrast, no difference (P = 0.645) in ADF digestibility was observed.

Although rumen liquor pH (Table 3) did not differ (P = 0.663) between the two dietary treatments, the total SCFA concentration was 50% greater (P = 0.005) in sheep fed at 2.0 x NE_m. The molar proportion of acetate was increased (P = 0.002) by 5.6% and that of propionate by 7.9% (P = 0.013) in sheep fed at 2.0 x NE_m, although the ratio of acetate to propionate production did not differ (3.52 days 3.55; P = 0.920). In contrast to the elevated levels of acetate and propionate in rumen liquor of growing days maintained sheep, the molar proportion of butyrate was 11% lower (P = 0.047). Similarly, sheep fed the 2.0 x NE_m diet possessed (numerically) lower concentrations of isobutyrate, isovalerate, and valerate.

Comparison of Plasma and Tissue Metabolite Concentrations

In growing animals, total SCFA plasma concentrations were 39% higher (P = 0.006) in growing (fed at 2.0 x NE_m) versus non-growing (fed at 1.2 x NE_m) animals, reflecting a 48% increase in acetate and a 100% increase (P = 0.022) in propionate concentrations (Table 4). In contrast, butyrate and isobutyrate concentrations did not differ, and 2-methylbutyrate and 3-methylbutyrate concentrations were below limits of detection (0.001 mmol/L).

With regard to glucose and gluconeogenic amino acids, no difference was detected between treatments for plasma concentrations of glucose, L-glutamate, and D-glutamate (Table 4). In total, D-glutamate accounted for 12.4% of the total plasma glutamate concentration in non-growing and 7.7% in growing sheep. In contrast to glutamate concentrations, the plasma glutamine concentration of growing animals was 28% greater (P = 0.009) than for non-growing, whereas alanine concentrations tended (P = 0.072) to be elevated by 22%.

To characterize potential differences between the concentrations of amino acids in tissues from growing versus non-growing animals and to identify potential relationships of plasma and tissue amino acid concentrations, the tissue concentrations of L-glutamate, D-glutamate, glutamine, and alanine were compared (Table 5). In contrast to plasma concentrations, no difference in alanine concentrations were observed between growing and non-growing animals. However, paralleling the increase in plasma glutamine concentrations, ileum epithelial glutamine concentrations (P = 0.135) tended to be greater in growing than non-growing animals. Between non-growing and growing animals, ileal D-glutamate and liver L-glutamate concentrations did not differ. However, the concentration of L-glutamate in ileal epithelia was 49% greater (P = 0.015) in growing versus maintained sheep, and the concentration of D-glutamate was 119% greater (P = 0.042) in liver of growing animals.

Table 2. Body weight and total tract digestion of lambs fed 1.2 or 2.0 x NE_m diets.[1,2]

	1.2 x NE_m	2.0 x NE_m	SEM	P Value
Item				
Initial BW (kg)	30.0[3]	30.0[4]	1.2	0.540
Final BW (kg)	29.0	33.0	1.4	0.001
Digestibility (g/100 g)				
DM	92.34	91.14	0.003	0.010
OM	92.63	91.16	0.003	0.002
CP	95.13	92.58	0.002	0.001
NDF	93.37	92.56	0.003	0.065
ADF	91.20	91.47	0.004	0.645
Energy	92.18	90.75	0.003	0.004

1 Values are means (n = 9) and pooled SEM from animals fed treatment diets for at least 14 days.

2 Abbreviations: BW = body weight; Ne_m = net energy of maintenance; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent.

3 Initial and final BW did not differ (SEM = 1.46; P = 0.139).

4 Initial and final BW differed (SEM = 0.61; P = 0.001).

Table 3. Rumen fermentation variables of lambs fed 1.2 or 2.0 x NE_m diets.[1,2]

	Treatment			
Item	**1.2 x NE_m**	**2.0 x NE_m**	**SEM**	**P Value**
pH	6.78	6.73	0.067	0.663
Total SCFA (mmol/L)	52.69	79.24	5.81	0.005
SCFA (moles/100 moles)				
Acetate	64.53	68.16	3.73	0.002
Propionate	18.56	20.03	1.54	0.013
Butyrate	6.66	5.96	0.397	0.047
Isobutyrate	5.07	2.80	0.213	0.152
Isovalerate	3.91	2.11	0.186	0.158
Valerate	1.29	0.98	0.078	0.517
Acetate:Propionate	3.52	3.55	0.169	0.920

1 Values are means (n = 9) and pooled SEM from animals fed treatment diets for at least 14 days. Rumen fluid samples were collected during the tissue collection protocol and analyzed for SCFA concentrations.

2 Abbreviations: Ne_m = net energy of maintenance; SCFA = short-chain fatty acids.

To characterize the relationship between glutamate isoform concentrations within tissues of non-growing and growing animals, the concentrations of L-glutamate and D-glutamate within ileal epithelial, liver, or kidney tissues were compared (Table 5). Within non-growing animals, the ileal epithelia contained about 47% more (P = 0.004) D-glutamate than L-glutamate (43.0 days 29.3 nmol/mg protein), whereas liver D-glutamate concentrations were only 16% that of L-glutamate (2.7 days 17.4 nmol/mg). Within growing animals, ileal epithelial concentrations of L- and D-glutamate (43.7 days 36.3 nmol/g) did not differ (P = 0.161), whereas liver D-glutamate concentrations were 36% that of L-glutamate (P = 0.001; 5.9 days 16.4 nmol/mg protein). In contrast to ileal and hepatic tissues, D-glutamate was not detected in kidney homogenates.

Table 4. Plasma metabolites of lambs fed 1.2 or 2.0 x Ne_m diets.[1,2]

	Treatment					
Item	**1.2 x NE_m**	**n**	**2.0 x NE_m**	**n**	**SEM**	**P Value**
Total SCFA (mmol/L)	0.67	9	0.93	9	0.056	0.006
Acetate (mmol/L)	0.65	9	0.90	9	0.056	0.006
Propionate (mmol/L)	0.009	9	0.020	9	0.002	0.022
Butyrate (mmol/L)	0.007	7	0.006	3	0.002	0.496
Isobutyrate (mmol/L)	0.005	5	0.004	3	0.003	0.414
2-Methylbutyrate (mmol/L)	bd[2]		bd			
3-Methylbutyrate (mmol/L)	bd		bd			
Glucose (mmol/L)	3.51	9	3.46	9	0.060	0.630
L-Glu (umol/L)	83.4	9	99.2	9	8.1	0.171
D-Glu (umol/L)	11.9	9	8.3	9	2.7	0.361
Gln (umol/L)	355.6	9	453.8	9	23.2	0.009
Ala (umol/L)	181.3	9	221.0	9	4.6	0.072

1 Values are means and pooled SEM from animals fed treatment diets for at least 14 days.

2 Abbreviations: NE_m = net energy of maintenance; bd = below detection; L-Glu = L-glutamate; D-Glu = D-glutamate; Gln = glutamine; Ala = alanine.

Comparison of GLT-1 and EAAC1 Protein Size and Quantity

Immunoblot analysis was performed on membrane proteins isolated from ileal epithelial, liver, and kidney tissues to compare relative levels of GLT-1 and EAAC1 expression by growing days non-growing sheep (Figure 1). Two predominant EAAC1 species (93 and 67 kDa) were detected in the ileum, liver, and kidney tissues of sheep of both treatment groups. For GLT-1, ileum epithelial and kidney tissues expressed predominant species of about 188 and 142 kDa. In addition, a third immunoreactive species of >203 kDa was expressed by liver tissue. No treatment effect in the size of immunoreactive species was observed. As qualitatively observed in autoradiographs (Figure 1) and quantitatively determined by densitometric analyses (Table 6), the amount of EAAC1 protein expressed by liver and kidney tissues between growing or non-growing sheep did not differ. In contrast, the ileal epithelia of growing sheep, which contained 49% more L-glutamate than did tissue from maintained sheep (Table 5), expressed 313% more (P = 0.04) EAAC1 protein than did ileal tissue isolated from the maintained sheep. For GLT-1, the relative amount of protein expressed by ileum epithelia or kidney collected from growing and non-growing sheep did not differ. However, the liver of growing sheep, which contained 119% more D-glutamate than did maintained sheep (Table 5), expressed 240% more (P = 0.001) GLT-1 protein than did sheep that maintained their weight over the experimental period.

Comparison of GLT-1 and EAAC1 mRNA Size and Quantity

To determine whether the differential expression of EAAC1 and GLT-1 protein was correlated with steady-state mRNA levels, Northern blot analysis was performed on RNA isolated from ileal and liver tissues (Figure 2). A single EAAC1 transcript of 2.8 kilobases (kb) was detected in the ileum of sheep from both dietary treatments, whereas a single GLT-1 transcript of about 12 kb was detected in liver tissue isolated from both groups of sheep. Equal loading and transfer of dietary treatment RNA to the blots was demonstrated by the detection of similar amounts of [^{32}P]-labeled 18S rRNA cDNA hybridization to 18S rRNA between treatment groups (Figure 2). In contrast to the greater expression of ileal epithelial EAAC1 and liver GLT-1 protein of growing animals, densitometric analyses demonstrated that the amount of EAAC1 (P = 0.79) or GLT-1 (P = 0.45) mRNA did not differ between dietary treatment groups (Table 7).

Table 5. Tissue amino acid concentrations of sheep fed 1.2 or 2.0 x NE_m diets.[1,2]

	Ileum				Liver				Kidney			
	1.2	**2.0**	**SEM**	**P Value**	**1.2**	**2.0**	**SEM**	**P Value**	**1.2**	**2.0**	**SEM**	**P Value**
L-Glu	29.3[3]	43.7[4]	2.5	0.015	17.4[5]	16.4[6]	2.0	0.723	40.3	32.0	5.8	0.368
D-Glu	43.0	36.3	2.4	0.116	2.7	5.9	1.0	0.042	bd	bd		
Gln	4.8	8.5	1.4	0.135	15.1	19.8	2.0	0.120	27.0	28.0	4.8	0.891
Ala	9.5	10.3	1.1	0.642	10.2	9.2	2.7	0.803	12.0	10.0	1.2	0.288

1 Values are means and pooled SEM of amino acid concentrations (nmols/mg protein) of ileum epithelial (n = 3), liver (n = 9), and kidney (n = 3) tissues collected from lambs fed treatment diets for at least 14 days.

2 Abbreviations: bd = below detection; Glu = glutamate; NE_m = net energy of maintenance.

3 L-versus D-glutamate differed (SEM = 1.60; P = 0.001).

4 L-versus D-glutamate did not differ (SEM = 3.02; P = 0.161).

5 L-versus D-glutamate differed (SEM = 1.51; P = 0.001).

6 L-versus D-glutamate differed (SEM = 1.66; P = 0.001).

Figure 1. Immunoblot analysis of EAAC1 and GLT-1 in ileal epithelium, liver, and kidney tissues of wether lambs fed 1.2 or 2.0 x NEm diets. Data are representative of 7 to 9 lambs evaluated for each tissue, as described in Table 6.

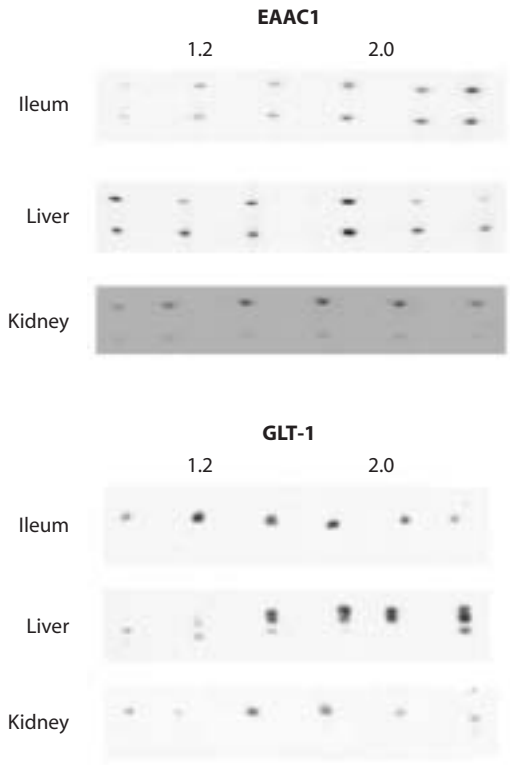


Table 6. Densitometric analysis of EAAC1 and GLT-1 expression of ileal epithelium, liver, and kidney tissues of sheep fed 1.2 or 2.0 x NE_m diets.[1,2]

	1.2 x NE_m	n	2.0 x NE_m	n	SEM	P Value
EAAC1						
Ileum	2.07	8	8.56	9	2.08	0.038
Liver	0.58	9	1.09	9	0.25	0.170
Kidney	0.81	8	0.91	9	0.26	0.776
GLT-1						
Ileum	4.44	7	6.18	9	1.54	0.412
Liver	0.65	8	2.21	9	0.24	0.001
Kidney	0.47	9	0.60	8	0.14	0.520

1 Values (arbitrary densitometric units) are means and pooled SEM from lambs fed treatment diets for at least 14 days. Ileum, liver, and kidney tissues were collected and analyzed. EAAC1 and GLT-1 expression by densitometric evaluation of immunoblot analyses.

2 Abbreviations: NE_m = net energy of maintenance.

Table 7. Densitometric analysis of EAAC1 and GLT-1 mRNA expression by ileal epithelium and liver tissues in sheep fed 1.2 or 2.0 x NE_m diets.[1,2]

	1.2 x NE_m	n	2.0 x NE_m	n	SEM	P Value
EAAC1						
Ileum	0.57	6	0.58	9	0.04	0.79
GLT-1						
Liver	2.15	7	1.79	6	0.35	0.45

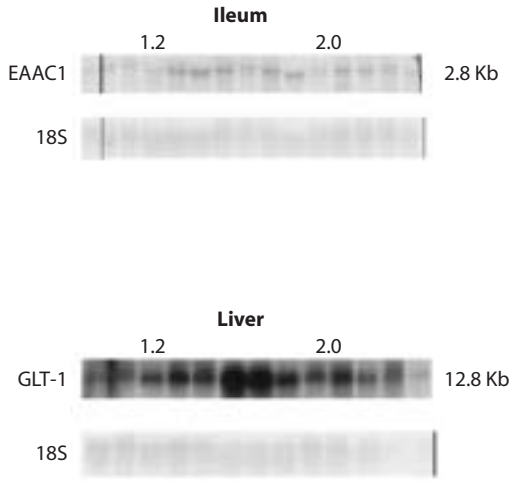
1 Values (arbitrary densitometric units) are means and pooled SEM. Lambs fed treatment diets for at least 14 days. Ileal epithelium and liver tissues were collected and analyzed by northern blot analysis for EAAC1 and GLT-1 mRNA expression as indicated.

2 Abbreviations: NE_m = net energy of maintenance.

As noted in the introduction, the metabolic fate of glutamate differs in small-intestinal epithelia, liver, and kidney of sheep. Given this knowledge, we were interested in determining whether the relative expression of EAAC1 and GLT-1 tissue content differed in lambs that were either growing (2.0 x NE_m treatment) or maintaining (1.2 x NE_m treatment) BW. Glutamate transporter expression was evaluated using this maintained-days-growing animal model, rather than a more extreme model of "starved-days-fed" adult sheep, to more accurately replicate typical physiological state/nutritional status of young, growing ruminants in production settings. These forage-based diets were formulated to yield equal quantities of protein (whether of dietary or microbial origin) to the small intestine and were similar to those used previously by this group to establish differences in growth of sheep. The use of these diets to establish growing days non-growing animal models was successful. Compared to non-growing animals, lambs fed 2.0 x NE_m achieved a moderate rate of growth, increased rumen and plasma concentrations of acetate and propionate, increased glutamine and alanine plasma concentrations but lowered total-tract diet digestibilities. Thus, the growing sheep possessed elevated nutrient metabolites and reduced digestibilities that are consistent with ruminants in a higher nutritional status. In addition, the numerically higher proportions of rumen liquor isoacids measured from non-growing sheep are indicative of lower fermentable carbohydrate supply. Overall, plasma L-glutamate concentrations reported in this study are comparable to what others have observed in the plasma of fed sheep. Although the plasma values do not take into account the glutamate contained in the red blood cells, the exchange of glutamate between plasma and blood cells is minimal.

Information regarding substrate regulation of System X^-_{AG} transporters is limited, especially with regard to whole-animal studies. A pertinent observation from this study was increased expression of EAAC1 by ileal epithelia and GLT-1 by liver tissue membranes of growing sheep. Coincident with increased glutamate transporter content was an increased concentration of L-glutamate in ileal and D-glutamate in hepatic homogenates. That EAAC1 content was elevated in ileal epithelia containing elevated L-glutamate whereas GLT-1 was not elevated indicates that in vivo EAAC1 expression may have been stimulated by the presence of L-glutamate whereas GLT-1 was not stimulated. In contrast to these results observed for ileal epithelial tissue, GLT-1 expression by primary "astrocyte-poor" neuronal cultures was increased in the presence of L-glutamate, whereas EAAC1 was not. An obvious difference between these experimental models is the influence of hormones. However, little is known regarding hormonal control of System X^-_{AG} transporter expression. Preliminary data from our lab (unpublished data) indicate that expression of System X^-_{AG} activity by isolated and fed steer hepatocytes is stimulated by insulin. Limited research with transgenic mice indicates that atypically high growth hor-

Figure 2. Northern blot analysis of 18S rRNA and EAAC1 mRNA isolated from the ileum or GLT-1 mRNA isolated from the liver of wether lambs fed 1.2 or 2.0 x NEm diets. Data are from 5 to 9 lambs evaluated for each tissue, as described in Table 7.



mone levels stimulate GLT-1 expression and that IGF-II is required for GLT-1 and EAAC1 expression.

A fundamental question of gene expression is where regulation occurs. In this study, post-transcriptional regulation likely occurred because increased expression of EAAC1 protein in ileal epithelial and GLT-1 protein (Table 6) in the liver of growing animals was not paralleled by increased mRNA levels (Table 7). Several other studies also indicate that steady-state levels of glutamate transporters do not correlate with mRNA levels. For example, increased amounts of EAAC1 protein expression by amino acid-deprived NBL-1 cells is accompanied by a decrease in EAAC1 mRNA levels. Similarly, the expression of EAAT4 mRNA in placental tissue is not coincident with protein expression. In contrast to our findings for GLT-1, increased expression of GLT-1 mRNA by primary astrocytes was paralleled by increased GLT-1 protein content when the medium was supplemented with dibutyryl-cAMP (dbcAMP). Along with the recent identification of membrane-binding proteins that separately regulate EAAC1 and EAAT4 activity, these data indicate that regulation of System X^-_{AG} proteins is complex and likely involves transcriptional, post-transcriptional, and post-translational regulation, depending on the particular effector, tissue, and/or species.

In the present study, sheep fed to gain were likely exposed to more digesta D-glutamate than non-growing sheep. That is, although the concentration of D-glutamate in digesta was not determined, the greater amount of microbial fermentation byproducts found in rumen fluid (Table 3) indicates that a greater amount of microbial protein, and hence D-glutamate, was presented to the small intestine for digestion and absorption. In terms of between-tissue comparisons of D-glutamate concentrations, a salient observation from the current study is that hepatic homogenates from growing sheep contained about twice as much D-glutamate than those from non-growing sheep (Table 5). Whether this reflects a relative saturation of hepatic oxidative capacity in animals with enough energy to grow, an enhanced expression of oxidizing capacity by animals of a nutritional status that did not support growth, or an increased capacity of growing animals to absorb D-glutamate remains to be determined.

In contrast to differences in hepatic D-glutamate concentrations, ileal epithelial homogenates did not differ, whereas no D-glutamate was detected in kidney homogenates of growing or non-growing sheep. For both growing and non-growing sheep, ileal concentrations were about 10 times higher than that measured in the liver. Accordingly, D-glutamate was relatively high in ileal, low in liver, and nonexistent in kidney tissue in both treatment groups. This ileal epithelia>liver>kidney D-glutamate concentration profile is consistent with D-glutamate concentrations measured in the tissues of nonruminants and is consistent with the concept that D-glutamate concentrations are inversely proportional to D-aspartate oxisdase activities. Because neither D-aspartate oxidase activity nor content was measured in the present study, it would be of interest to determine in future studies if small-intestinal epithelia of young sheep express a lower capacity to metabolize D-glutamate, as appears to be the case for nonruminants.

With regard to inter-species comparison of D-glutamate concentrations, concentrations of 36.9 and 138 nmol/g tissue have been measured in adult male rat liver and kidney, respectively. Because these data were reported as nmol/g tissue, values from rat and sheep cannot be directly compared. However, if it is assumed that 1 g of tissue contains 10% protein, then D-glutamate concentration in sheep and rat liver can be compared by using a coefficient factor of 100:

nmol/mg protein * 1000 mg protein/g protein * 0.1g protein/g tissue = 100 nmol/g tissue.

Applying this conversion, sheep liver from the present study contained 270 to 590 nmol D-glutamate/g, which is two to four times the concentration of D-glutamate in rat liver. That levels of D-glutamate (138 nmol/g) in rat kidney exceeded that of rat liver whereas D-glutamate was not detected in sheep kidney also suggests that fundamentally different levels of D-glutamate supply and/or capacity for metabolism exist between ruminant and nonruminant species. That is, animals exposed to large amounts of D-glutamate in the digesta (e.g., ruminants) likely have increased capacities to metabolize D-glutamate. Nonruminant intestinal epithelia are not considered to have large, if any, capability to metabolize D-isomers. Instead, D-isomers are metabolized by hepatic and renal enzymes. That D-glutamate concentration in sheep ileal epithelia was highest compared to liver and kidney indicates that the intestinal epithelia of ruminants also possess relatively low D-glutamate metabolic capacity.

In summary, the expression of two System X^-_{AG} glutamate transporters (EAAC1, GLT-1) and D- and L-glutamate concentrations was evaluated in lambs that gained or maintained body weight on forage-fed diets. Measurements of rumen liquor and plasma metabolites reflected a higher nutritional status of growing animals. The content of both EAAC1 and L-glutamate was increased in ileal epithelia of growing days non-growing wethers, whereas in liver, GLT-1 and D-glutamate content was in-

creased. In contrast, no differences in transporter isoform expression were observed in the kidney, coincident with no differences in either glutamate isomer concentration. These results indicate that the differential expression of EAAC1 and GLT-1 system X^-_{AG} transport proteins may be linked to the different metabolic functions of these tissues.

Summary

Forage-fed cattle of Kentucky often are supplied with an excess of protein but a deficit of energy. During these periods, a portion of the excess protein can be metabolized to support energy needs. Therefore, the overall goal of this research is to understand how dietary and bacterial proteins are metabolized to support tissue-specific energy requirements. The intestine preferentially metabolizes (oxidizes) the amino acids glutamate and glutamine (after deamination to glutamate) as non-glucose energy sources, thus sparing absorbed glucose for use by the rest of the body. Of all the amino acids, glutamate is the most extensively oxidized. For example, about 43% of total glutamate in forage-fed lactating dairy cattle is oxidized to CO_2, a level greater than that observed for aspartate, alanine, and glutamine (about 28% to 35%). Overall, these extensive oxidation values approximate those observed for acetate, propionate, and butyrate, which are considered the principal sources of metabolic energy in ruminants.

For glutamate to be metabolized, it first must be absorbed (transported) across cellular membranes. Previously, we identified two different proteins (EAAC1 and GLT-1) in the cellular membranes of intestine, liver, and kidney tissues of sheep and cattle that are capable of absorbing glutamate. Therefore, the immediate goal of the present research was to determine the relationship between concentrations of glutamate (both L- and D-isomers) in plasma and tissue (ileal epithelium, liver, or kidney) and the amount of EAAC1 and/or GLT-1 present in the tissues of wethers that maintained or gained (0.6 lbs/day) body weight. L-glutamate, D-glutamate, glutamine, and alanine concentrations were measured in blood plasma and tissue homogenates. Although there was 27.6% more glutamine in the plasma of growing animals, plasma concentrations of D-glutamate, L-glutamate, and alanine did not differ.

With regard to tissue concentrations of glutamate, there was 49% more L-glutamate in ileal epithelia, and 181% more D-glutamate in liver tissue, of growing days non-growing animals. In contrast, no differences in renal tissue concentrations of glutamate were observed. With regard to the amount of proteins capable of absorbing glutamate, 313% more EAAC1 protein was found in ileal tissues of growing sheep days non-growing sheep, whereas liver content of EAAC1 did not differ. In contrast, 240% more GLT-1 protein was expressed by the liver of growing sheep, whereas ileal expression did not differ. As for EAAC1, kidney tissue content of GLT-1 of growing animals and non-growing animals did not differ. These results indicate that the expression of EAAC1 and GLT-1 is differentially modulated by growth state and/or tissue concentrations of L- or D-glutamate.

Future work will focus on describing how the relationships that exist between tissue concentrations of L- and D- glutamate, glutamate transporters, and glutamate-metabolizing enzymes putatively differ to support the growth of cattle at various stages of their life cycle.

Effect of Supplemental Energy Source on Growth and Reproductive Performance of Virgin Heifers Consuming Corn-Silage-Based Diets

C.M. Howlett, E.S. Vanzant, L.H. Anderson, W.R. Burris, J. Randolph, and R.F. Bapst

Introduction

There is currently much interest in potential effects of dietary fat and total dietary energy on reproductive performance of cattle. However, most of the studies have used multiparous cows and little information has been gathered with virgin heifers as a model. Additionally, few studies have actually looked at reproductive performance (e.g., conception rates). Most of the research has concentrated on ovarian function and hormone levels. Our goal was to evaluate the effects of dietary treatments on conception rates of virgin heifers on corn-silage-based diets.

The form of energy supplied in the diet not only has an effect on digestive and metabolic properties, but also, as a consequence, it can have an effect on a variety of performance characteristics. Previous research has suggested that addition of fats or oils to the diet can have a positive effect on ovarian function, even when total dietary energy intake is unaffected. However, inclusion of fat in the diet at a level greater than 5% of the dry matter intake can have a detrimental effect on rumen microbial function, which can decrease digestibility and reduce weight gains.

A major difficulty in animal production schemes is accurate prediction of animal performance. While energy values of supplements are fairly consistent, forage energy values tend to fluctuate. Typically, energy values of forages are predicted empirically from acid detergent fiber values. However, summative approaches—which incorporate neutral detergent fiber, lignin, protein, ether extract, and acid detergent insoluble nitrogen (unavailable protein) values—have been suggested to be more accurate with some forages.

The objectives of this study were to evaluate the effect of altering dietary energy source on weight gains and reproductive performance of heifers grown on corn-silage-based diets and to determine the accuracy of using a summative approach to calculate the net energy of corn silage.

Procedures

Ninety-six crossbred, virgin beef heifers (249 kg; 550 lb) were used in a randomized complete block design to determine the effects of source of supplemental nutrients on average daily gain and reproductive performance. Heifers were blocked by weight and randomly assigned to 12 pens of eight heifers each. Pens within blocks were randomly assigned to receive one of four diets based on corn silage (CP = 8.2%; TDN = 63.5%; NE_m = 1.42 Mcal/kg or 0.64 Mcal/lb) at 42% of the dry matter intake. Treatments consisted of the following supplements fed at the specified percentage of dry matter intake:

- corn and soybean meal, 56%;
- whole linted cottonseed, 15%;
- whole raw soybeans, 15%; or
- pelleted soybean hulls, 30%.

In Diets 2 through 4, cottonseed, soybeans, and soybean hulls replaced a portion of the corn and soybean meal. An inadvertently low net energy (NE) value for soybean hulls was used in the formulation of the diets, which resulted in a higher feeding level for the soybean hulls treatment relative to the other treatments. Diets were formulated to be isonitrogenous (13.7% CP) and were fed at approximately 2.2 x NE_m (with the exception of soybean hulls, which were fed at approximately 2.8 x NE_m) to achieve target weights equal to 65% of expected mature body weight at the time of artificial insemination (AI).

Animals were weighed every 28 days for 112 days with weights obtained on consecutive days at the beginning and end of the treatment period. After each weighing, feeding levels were adjusted for each pen to account for weight gain and associated changes in maintenance energy requirement. Beginning on Day 113, treatments were discontinued, and all groups were fed a common diet at an appropriate level to maintain target gains. Serum was obtained for progesterone analysis on Days 112 and 119. Heifers were considered to have initiated estrous cycles if progesterone concentrations exceeded 0.5 ng/ml in either sample. Heifers were synchronized with an MGA/prostaglandin system and bred by AI in response to detected heat on Days 154 to 156 (beginning 48 hours after prostaglandin administration).

Statistical Analysis

The GLM procedure of SAS was used to identify significant treatment effects for heifer weight gains. Means were separated by a protected ($P < 0.10$) Fisher's LSD. For the ovarian function and pregnancy data, a chi-square analysis of binomially distributed data was performed.

Results and Discussion

Because the energy value for soybean hulls was underestimated, cumulative average daily gain for soybean hulls (1.02 kg/day; 2.25 lb/day) was greater ($P \leq 0.03$) than for corn/soybean meal (0.88 kg/day; 1.94 lb/day), whole linted cottonseed (0.87 kg/day; 1.91 lb/day), or whole raw soybeans (0.86 kg/day; 1.90 lb/day). Using current book values for the energy concentration of all supplements, including soybean hulls, and a summative approach for calculating the energy content of corn silage, heifer gains were accurately predicted.

For the reproductive data, the variables of interest were number of heifers that were pubertal and conception rates of heifers on the various treatments. Although some numerical trends existed, no significant treatment effects were detected for reproductive characteristics. Percentages of pubertal heifers prior to synchronization for each treatment were: corn/soybean meal (60%), whole linted cottonseed (53%), whole raw soybeans (69%), and soybean hulls (71%). First-service conception rates were: corn/soybean meal 7:19 (37%), whole linted cottonseed 8:21 (38%), whole raw soybeans 12:22 (55%), and soybean hulls 8:19 (42%).

All of the experimental diets resulted in acceptable growth performance of the heifers in this study. First-service conception rates were below expectations, possibly due to abnormally warm and humid conditions during breeding. Maximum ambient temperatures at the time of AI ranged from 29 to 31°C, with relative humidities ranging from 42 to 100%. At this location, average monthly temperatures for the same time period were greater in only 15 of the last 100 years. Although our treatments caused some numerical shifts in percentage of pubertal heifers and first-service conception rates, these effects were not statistically significant. Others have reported variable effects of fat supplementation on reproductive performance of beef females, particularly with yearling heifers. Research suggests that linoleic acid supply may be an important mediator of reproductive effects of fat. Thus, one factor that may affect responses to fat supplementation is effects of ruminal microbial activity on flow of linoleic acid out of the rumen. Additional research is under way to determine effects of these diets on flow of nutrients to the small intestine.

Summary

This research suggests that our ability to alter reproductive performance of virgin beef heifers by using oilseeds as a dietary fat source may depend on the nature of the diet consumed by the heifers. We used 96 crossbred virgin heifers to determine the effect of source of supplemental nutrients on heifer reproductive performance and average daily gain. We added supplements of corn/soybean meal, whole raw soybeans, whole linted cottonseed, or pelleted soybean hulls to corn silage and fed once daily. We did not significantly alter the proportion of heifers that were pubertal at the beginning of the breeding season or conception rates to artificial insemination. Other studies have shown inconsistent effects of dietary fat on reproductive performance of beef females. We believe that some of this variation may be related to factors associated with the diet that can affect how fatty acids are altered within the ruminal environment. With all of our diets, heifer weight gains were accurately predicted by net energy equations using NRC (1996) values for energy concentrations of supplements and a summative approach for calculating the net energy concentration of corn silage.

Effects of Early Weaning versus Conventional Weaning on Calf Weaning Weight, Average Daily Gain, Intake, and Diet Digestibility

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Introduction

Early weaning of beef calves, especially during drought conditions, has improved performance of both the dam and the calf. Early weaning has increased cow reproductive efficiency (increased pregnancy rates and decreased postpartum interval) and performance (less weight loss and maintenance of more body condition during lactation). Weaning earlier than the typical 210 days of age and placing calves in drylot have also increased average daily gain (ADG) and feed efficiency in calves. However, placing early-weaned calves in drylot is expensive, due to increased feed costs, and may not be feasible for producers living in the eastern United States, where forage is typically abundant and feedyards are less numerous than in other regions of the United States. Therefore, the objective of this study was to determine the effects of early weaning in tall fescue pastures with supplemental feed on calf weight, ADG, intake and digestibility, cow weight change, and body condition score change.

Procedures

Sixteen Angus x Beefmaster cows and their calves were allotted to two treatments from May 26 to September 21, 1999. Treatments included:

- early weaned (~120 days of age) calves creep fed (1 $kg \cdot hd^{-1} \cdot d^{-1}$ for 3 weeks) on tall fescue pastures with continued supplementation (3.6 $kg \cdot hd^{-1} \cdot d^{-1}$) after weaning, and
- normal-weaned (~210 days of age) on tall fescue pastures without creep or supplemental feed.

Prior to early weaning and within each treatment group, calves and their dams were maintained in two 1.4-ha, endophyte-infected tall fescue (*Festuca arundinacea*, Kentucky 31) pastures. At the time of early weaning (EW; June 30), dams were separated from their calves and moved to a pasture adjacent to the calf pasture where they remained for two weeks. On July 15 early-weaned calves and normal-weaned (NW) calves and their dams were assigned to eight 1.4-ha, endophyte-infected tall fescue pastures with two calves per pasture (one steer and one heifer) and four pastures per treatment. Dams with early-weaned calves were maintained as one herd in a pasture distant from their calves.

Calf and cow weights and cow body condition scores (BCS; scale 1 to 10) were recorded every two weeks. Other measurements included calf ADG, cow weight change, and BCS change.

Calf dry matter intake (DMI) and dry matter digestibility (DMD) were estimated twice (Periods 1 and 2) during the trial using a chromium oxide time-release bolus as an external indicator of digestibility. Fecal grab samples were collected on Days 10 through 14 following introduction of boluses.

Milk consumption was measured during each of the digestibility periods for each calf within the normal weaning treatment group using weigh-suckle-weigh. This, along with forage intake estimations from boluses, was used to determine DMI and DMD for the normal-weaned calves. Average intake of the growing diet during each digestion period, along with forage intake estimations from chromium oxide boluses, was used to determine DMI and DMD for early-weaned calves (assuming equal consumption between both calves within each pasture).

Results and Discussion

Cow and Calf Measurements

Calf weights differed throughout the trial except at start of creep feeding and Period 1. At early weaning, early-weaned calves weighed 16.4 kg more (Table 1; $P < 0.1$) than normal-weaned calves due to the early-weaning calves having received a creep diet for nearly two weeks prior to weaning. At normal weaning (September 21), early-weaning calves were 21.9 kg heavier than normal-weaned calves; however, 205-day adjusted weaning weights were not different.

Average daily gains were greater ($P < 0.01$) for early-weaned calves while they consumed a creep diet and milk from their dams (1.1 versus 0.8 kg/d; Table 2). However, immediately following early weaning and until time of pasture allotment, normal-weaned calves had greater ADG ($P < 0.03$) than early-weaned calves (0.9 days 0.5 kg/day; Table 2). Slower gains of early-weaned calves during this period were the result of weaning. Once all calves were assigned to their pastures (after July 15), gains did not differ for the remainder of the trial.

Weights and BCS of cows were not different between treatments when the trial was initiated on May 26. No differences were detected in weight change from the start of creep feeding (May 26) to early weaning (June 30). From June 30 to September 21, cows in the early-weaned treatment gained 35.2 kg each, while those in the normal-weaned treatment lost 7.7 kg/hd.

Cows with early-weaned calves also gained more condition from June 30 to September 21 than those with new calves (1.72 versus 0.19; $P < 0.01$).

Intake and Digestibility Measurements

Dry matter intake was higher ($P < 0.01$) in Period 1 for early-weaned calves (4.0 $kg \cdot hd^{-1} \cdot d^{-1}$; Table 5) than for normal-weaned calves (3.4 $kg \cdot hd^{-1} \cdot d^{-1}$). Intake was also higher for early-weaned calves in Period 2, ($P = 0.03$), (4.3 versus 3.3 $kg \cdot hd^{-1} \cdot d^{-1}$, respectively). The diet of early-weaned calves (supplement and fescue forage) was more digestible (DMD) than the diet for normal-weaned calves (milk and fescue forage) in both Periods 1 and 2.

Nutrient digestibility (CP and NDF) of the total diet was also measured during Periods 1 and 2. Crude protein digestibility did not differ between early- and normal-weaned calves in Period 1 but was more ($P = 0.01$) digestible in early-weaned calves during Period 2 (66.0 versus 50.8%). Neutral detergent fiber digestibility was greater in early-weaned calves in Periods 1 ($P < 0.05$) and 2 ($P < 0.01$).

The consumption of a highly digestible supplement, in addition to forage, by early-weaned calves may have contributed to treatment differences. On the other hand, normal-weaned calves were consuming very little milk by the end of the study (2.7 $kg \cdot hd^{-1} \cdot d^{-1}$ at end of trial versus 9.0 $kg \cdot hd^{-1} \cdot d^{-1}$ at start of trial).

Summary

Sixteen Angus x Beefmaster calves were assigned to be creep fed and early-weaned on tall fescue pastures at approximately 120 days of age or to be normal-weaned (~210 days) on tall fescue pastures without creep feed. Calf weights and ADG, cow body weight change, and BCS change were measured at various times throughout the trial. Dry matter intake and DMD were measured using steers in both treatment groups. Milk consumption was measured in normal-weaned calves using weigh-suckle-weigh, and supplement consumption was measured in early-weaned calves.

Milk and supplement consumption were used in part of an equation to determine DMI. Nutrient digestibility of the total diet was also analyzed for both treatment groups. Adjusted 205-day weaning weights were similar between treatment groups.

Average daily gains were greater for early-weaned calves from the start of creep feeding (May 26) to early weaning (June 30) but were higher for normal-weaned calves from early weaning up to the start of Period 1 (July 15). Dry matter intake and DMD were greatest for early-weaned calves during both periods. Crude protein digestibility was similar for both treatment groups in Period 1 but was greater for early-weaned calves during Period 2. Neutral detergent fiber (NDF) digestibility was higher for early-weaned calves during both Periods 1 and 2. Early weaning calves at 120 days of age did not improve animal performance above that of calves weaned at 210 days of age but did result in greater dry matter consumption and increased digestibility of dietary fiber.

Table 1. Body weights (kg) of early-weaned (EW) and normal-weaned (NW) calves from May 26 to September 21, 1999.

Date	EW	NW	P Value
May 26[a]	124.8	120.0	NS
June 30[b]	164.8	148.4	0.07
July 15[c]	172.5	162.2	NS
Aug. 11	200.9	184.1	0.09
Sept. 21[d]	239.6	217.7	0.08
Adjusted 205-d WWt.	243.2	228.7	NS

[a] Initiation of creep feeding to EW calves.
[b] Early weaning.
[c] Assignment to pastures.
[d] Normal weaning.

Table 2. Average daily gains (kg) of early- and normal-weaned calves.

Date	EW	NW	P Value
May 26 to June 30[a]	1.1	0.8	0.01
June 30 to July 15[b]	0.5	0.9	0.03
July 15 to Aug. 11 (Period 1)	1.0	0.8	NS
Aug. 12 to Sept. 21 (Period 2)	0.9	0.8	NS
Total Trial			
June 30 – Sept. 21[c]	0.9	0.8	NS
July 15 – Sept. 21[d]	1.0	0.8	NS

[a] Start of creep feeding to EW calves until early weaning.
[b] Early weaning to placement in pastures.
[c] Early weaning to normal weaning.
[d] Placement in pastures after early weaning until normal weaning.

Table 3. Average weight change (kg) of cows with early- and normal-weaned calves.

Date	EW	NW	P Value
May 26 to June 30[a]	-7.6	-5.4	NS
June 30 to July 15[b]	-1.4	33.3	0.09
July 15 to Aug. 11 (Period 1)	20.7	-42.7	0.01
Aug. 12 to Sept. 21 (Period 2)	15.9	1.7	0.05
Total Trial			
June 30 – Sept. 21[c]	35.2	-7.7	0.01
July 15 – Sept. 21[d]	36.6	-41.0	0.01

[a] Start of creep feeding to EW calves until early weaning.
[b] Early weaning to placement in pastures.
[c] Early weaning to normal weaning.
[d] Placement in pastures after early weaning until normal weaning.

Table 4. Body condition score change of dams with early- and normal-weaned calves.[a]

Date	EW	NW	P Value
May 26 to June 30[b]	-0.42	-0.41	0.06
June 30 to July 15[c]	0.77	0.26	0.09
July 15 to Aug. 11 (Period 1)	-0.07	-0.67	0.05
Aug. 12 to Sept. 21 (Period 2)	1.02	0.61	0.04
Total Trial			
June 30 – Sept. 21[d]	1.72	0.19	0.01
July 15 – Sept. 21[e]	0.95	-0.06	0.01

[a] 1 = emaciated; 10 = obese.
[b] Start of creep feeding to EW calves until early weaning.
[c] Early weaning to placement in pastures.
[d] Early weaning to normal weaning.
[e] Placement in pastures after early weaning until normal weaning.

Table 5. Dry matter intake and digestibility of diets consumed by early- and normal-weaned calves grazing tall fescue pastures.

	EW[a]	NW[b]	P Value[c]
Total DMI, $kg \cdot hd^{-1} \cdot d^{-1}$			
Period 1 (July 15 – Aug. 11)	4.0	2.91	0.01
Period 2 (Aug. 12 – Sept. 21)	4.3	3.3	0.03
Total DMD, %			
Period 1 (July 15 – Aug. 11)	67.0	52.9	0.01
Period 2 (Aug. 12 – Sept. 21)	61.9	40.8	0.01

[a] Growing supplement included in calculation for total DMI and DMD.
[b] Milk consumption included in calculation for total DMI and DMD.
[c] Observed significance level.

Table 6. Crude protein (CP) and neutral detergent fiber (NDF) digestibility by early- and normal-weaned calves grazing tall fescue pastures.

	Period 1			Period 2		
Nutrient	**EW**	**NW**	**P Value**	**EW**	**NW**	**P Value**
CP, %	64.7	62.8	NS	66.0	50.8	0.01
NDF, %	62.1	35.2	0.04	58.9	32.2	0.01

Quality Evaluation of Case-Ready Beef Steaks from Various USDA Grades

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Introduction

Case-ready packaging technologies have been shown to improve shelf life and maintain quality of fresh ground meat. However, few studies have focused on whole-muscle beef cuts. With the need for increased safety of our food supply, the meat industry has attempted to minimize contamination potential by eliminating cross-contamination as well as mishandling at the retail store level with case-ready products.

Our objectives in this study were to evaluate the chemical and the visual benefits of high oxygen (80% O_2/20% CO_2) modified atmosphere packaging (MAP) on whole-muscle round steaks with different USDA quality grades.

Procedures

Nine carcasses were selected, based on USDA Quality Grades (three Select, three Low Choice, and three High Choice). Rounds were obtained and fabricated into three major muscles (semitendinosus, semimembranosus, and biceps femoris). Each muscle was cut in half and allocated to modified atmosphere packaging (MAP) or polyvinyl chloride overwrap (PVC). One-half of each muscle was sliced into 2.54 cm steaks and packaged in high oxygen (80% O2/20% CO2) MAP and allotted to storage of one, three, five, seven, and 10 days. The other half was sliced into 2.54-cm steaks and packaged with an oxygen-permeable PVC overwrap and allotted to storage of one, three, five, seven, and 10 days.

Steaks were visually evaluated on Days 1, 3, 5, 7, and 10 for lean color (8 = bright cherry red; 1 = extremely brown or green), surface discoloration (11 = 0% discoloration; 1 = 90% to 100% discoloration), and overall appearance (8 = extremely desirable; 1 = extremely undesirable).

A Minolta Chroma Meter CR-300 colorimeter (L*, a*, and b* values) was used to objectively examine color on Days 0, 1, 3, 5, 7, and 10. Metmyoglobin content was determined using the procedure described by Krzywicki (1982). Beef steaks were analyzed in duplicates for the 2-thiobarbituric acid reactive substances (TBARS) at each storage time to provide a measure of lipid oxidation within each treatment, as described by Witte et al. (1970). These data were analyzed utilizing a factorial arrangement with 2 Packaging (MAP and PVC) x 3 Grade (USDA High Choice, Low Choice, and Select) x 3 Steak (semimembranosis, semitendinosis, and biceps femoris) x 5 Storage Time (one, three, five, seven, and 10 days).

There were no USDA grade x packaging treatment interactions ($P > 0.05$) for lean color, discoloration, overall appearance, or L*, a*, and b* values. However, USDA grade effect for these quality parameters was significant, with Select (41.125) and Low Choice (41.077) being more desirable than High Choice (39.160) for L* values and Low Choice being more desirable than both High Choice and Select for lean color (6.4, 5.8, and 5.9, respectively), discoloration (8.7, 7.5, and 7.9, respectively), overall appearance (6.1, 5.5, and 5.6, respectively), and a* (13.891, 12.086, and 12.759, respectively) and b* (5.334, 4.858, and 5.139, respectively) values. Correale et al. (1986) found surface discoloration and overall appearance scores of USDA Prime and Choice steaks packaged in high oxygen barrier film often were more desirable ($P < 0.05$) than those of USDA Good (Select) steaks. Kennick and associates (1971) determined degrees of marbling had a significant curvilinear effect on the case life of fresh steaks, with slight (Select) and slightly abundant (Prime) amounts of marbling being least desirable. In addition, Kennick et al. (1971) stated the small, modest, and moderate degrees of marbling, which are typical of USDA Choice beef, had longer case life.

There were no grade x packaging interactions for percent metmyoglobin and TBARS values; however, grade had a main effect ($P < 0.05$) on percent metmyoglobin content, with High Choice being higher than both Select and Low Choice (32%, 26%, and 25%, respectively). TBARS values also differed ($P < 0.05$) among grades, with Low Choice steaks being more desirable. Correale et al. (1986) found differences in metmyoglobin formation among strip steaks from Prime, Choice, and Good grades due to inherent characteristics of the muscles. Muscle fibers from Prime and Choice samples were redder, while muscle fibers of Good (Select) samples were whiter.

These findings indicate quality grade has a major influence on color stability of high oxygen packaged beef steaks. Regardless of muscle type and grade, however, whole-muscle steaks from the round can achieve an extended shelf life by use of novel MAP technology.

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Methods for Measuring Indigestible ADF as a Digestion Marker for Fescue-Based Diets

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Introduction

In order to measure intake and digestibility of forage by grazing animals, nutritionists often use internal markers and indigestible components of feeds, in combination with estimates of fecal output. A variety of factors, including forage type, can affect the usefulness of internal markers, and it is, therefore, necessary to validate the use of a given marker technique with each forage type for which it is intended to be used. Generally, validation involves feeding a known amount of a forage in confinement, collecting all fecal output, measuring the amount of marker consumed and excreted, and calculating fecal recovery of the marker as a percentage of that consumed.

Previously (*2000 Kentucky Beef Cattle Research Report*) we reported near 100% recoveries of indigestible acid detergent fiber (IADF) from steers consuming a moderate-quality fescue hay. Other markers evaluated had either high (110% for acid detergent lignin) or low (90% for acid-insoluble ash) recoveries. However, compared with other markers, standard techniques for measuring IADF are laborious and time-consuming. These techniques involve individually weighing samples into test tubes, collecting ruminal fluid from a fistulated animal, and individually administering measured quantities of ruminal fluid and buffer to the individual tubes. This is followed by long-term (96 to 144 hours) incubation and then by boiling in acid detergent solution and filtration through filtering crucibles.

New technology recently has become available that allows for *in vitro* fermentation and subsequent fiber measurement within porous nylon bags (Ankom Corp., Fairport, N.Y.). Using this technology may make it possible to eliminate some of the more tedious steps in IADF isolation from samples. The objective of this experiment was to evaluate modified IADF isolation procedures with respect to the effectiveness of IADF as an internal marker. Specifically, we were interested in comparing two different materials for sample bag construction and two incubation techniques with the standard approach described above.

Procedures

We used samples that were collected in a previous experiment designed to evaluate several different internal markers. In that experiment, four Angus steers (average body weight = 440 kg; 970 lb) were fed a 100% fescue hay diet (15% CP; 77% NDF; 43% ADF; 4.8% ADL) at 95% of ad libitum intake. Indigestible ADF was measured using five different procedures (Table 1). All IADF procedures included acid/pepsin pretreatment of forage samples, which has previously been shown to improve fecal recoveries. For standard IADF (STD), samples were incubated *in vitro* individually for 144 hours, extracted in acid detergent solution, and filtered through coarse Gooch crucibles. The other four procedures were arranged as a 2 x 2 factorial: batch incubation of samples for 168 hours, either intraruminally (*in situ*; IS), or in an Ankom batch *in vitro* incubator (IV), in either standard *in situ*-type bags (Ankom # 1020) cut to 5.0 by 5.5 cm and heat sealed, or fiber filtration bags (Ankom #F-57; 5.0 by 5.5 cm), followed by batch acid detergent extraction.

Indigestible ADF concentrations in hay and fecal samples were analyzed as a completely randomized design with the GLM procedure of SAS with a model appropriate for a 2 x 2 x 2 + 1 factorial arrangement of treatments. In this case, factors included sample type (hay versus feces), bag construction (F-57 versus 1020), and incubation method (*in situ* versus *in vitro*), and the final treatment included the standard IADF procedure. Fecal recoveries of IADF were also subjected to analysis of variance using the GLM procedure of SAS with a model appropriate for a 2 x 2 + 1 treatment structure (same as described above with the exclusion of sample type). Because we knew, a priori, that the fecal recovery for the standard procedure was essentially 100%, contrasts constructed to compare each of the alternative methods with the standard procedure allowed:

- comparison with the standard procedure, and
- determination of whether recovery values differed significantly from 100%.

Table 1. Detail of the five IADF procedures evaluated.

		In situ		*In vitro*	
	Standard	**Ankom 1020 *in situ* bags[1]**	**Ankom F57 filter bags**	**Ankom 1020 *in situ* bags[1]**	**Ankom F57 filter bags**
Technique abbreviation	STD	IS-1020	IS-F57	IV-1020	IV-F57
Pre-incubation of hay in acid-pepsin	Yes	Yes	Yes	Yes	Yes
Sample size, g	0.5	0.5	0.5	0.5	0.5
Incubation type	Individual sample	Bulk	Bulk	Bulk	Bulk
Incubation vessel	Glass culture tube	Rumen	Rumen	Ankom Daisy$^{II®}$ batch incubator	Ankom Daisy$^{II®}$ batch incubator
Incubation time	144 h	168 h	168 h	168 h	168 h
ADF analysis	Individual, Gooch crucible	Batch, Ankom fiber analyzer	Batch, Ankom fiber analyzer	Batch, Ankom fiber analyzer	Batch, Ankom fiber analyzer
Replicates[2]	3	3	3	3	3

[1] Bags (10 x 20 cm) were cut to similar size as F-57 bags (approx. 5.0 x 5.5 cm).

[2] Side-by-side replicates for STD procedure; replicates analyzed in separate incubations in bulk procedures.

Results and Discussion

IADF concentrations of hay and fecal samples were affected by a sample type x treatment interaction (Table 2); differences between techniques were greater with fecal than with hay samples, although ranking of the techniques was similar between sample types. For fecal samples, bag type had a larger influence (incubation procedure x bag type; P = 0.02) on IADF concentrations when samples were incubated in situ rather than *in vitro*, whereas the interaction was not significant (P = 0.42) for hay samples.

Fecal recoveries differed from STD for IS/1020 and IS/F57 but not for IV/1020 or IV/F57. However, all techniques resulted in fecal recoveries that were within acceptable ranges (96.2% to 107.5%) for generating intake and digestibility estimates with a reasonable degree of accuracy. Furthermore, very little difference existed among the techniques in the variation between replicate analyses on the same sample. Standard deviations of IADF concentration within sample averaged 1.5%, 2.6%, 3.4%, 2.0%, and 1.8% for STD, IS/1020, IS/F57, IV/1020, and IV/F57, respectively. Likewise, standard deviations (between animals) for fecal recoveries averaged 4.2%, 5.9%, 8.4%, 8.0%, and 4.8% for these treatments.

Summary

Despite differences in absolute amount of IADF measured in hay and fecal samples compared with a standard method for measuring IADF, using bulk *in vitro* incubation with either Ankom #1020 or Ankom #F57 polyester bags resulted in acceptable fecal recoveries for cattle consuming fescue hay. Although fecal IADF recoveries differed from 100% when in situ incubations were used with the two bag types, differences were relatively small, as were differences in variation in IADF measurement among the procedures.

Small differences among the approaches need to be weighed against savings in labor and time when considering which approach to use. With fescue hay, any of the IADF measurement techniques yielded reasonable fecal recoveries. Additional validation of these techniques is required before the approach is adopted with other forages.

Table 2. IADF concentrations of fescue hay and fecal samples by different procedures.[a]

Procedure	Hay IADF,[b] %	Fecal IADF,[c] %
STD	17.4	37.4
IS-1020	10.4	23.5
IS-F57	14.8	34.2
IV-1020	13.4	27.9
IV-F57	16.7	34.6
SE	.9	.6

[a] Sample type x technique (P < 0.01).
[b] Incubation procedure x bag type (P = 0.42).
[c] Incubation procedure x bag type (P = 0.02).

Table 3. Fecal recoveries from different IADF procedures.

Procedure	Fecal Recovery, %	Contrast P Value[a]
STD	100.1	
IS-1020	105.6[a]	0.04
IS-F57	107.5[a]	0.01
IV-1020	97.1	0.23
IV-F57	96.2	0.12
SE	1.7	

[a] Single degree of freedom contrasts of STD versus other procedures.

The Use of Multispectral Radiometry and Neural Network Technology to Predict Standing Forage Biomass of Fescue-Based Pastures

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Introduction

The ability to measure standing forage biomass of pastures is important for managerial practices such as fertilizer application and adjusting stocking rates. However, problems associated with methods used to determine forage production have limited the use of this type of information. Currently, the most reliable biomass estimations are obtained from hand-clipped sampling techniques that are labor-intensive and destructive. A tool that would provide quick and nondestructive predictions of standing forage biomass across a large area would benefit producers by limiting pasture damage or losses in performance caused by over- or undergrazing. Also, this type of tool could be valuable for use in precision agriculture systems by allowing for development of "yield" maps to facilitate area-specific fertilizer application.

New remote sensing technologies, such as multispectral radiometry (MSR), have the potential to provide inexpensive, rapid, and abundant estimates of standing forage biomass. The basis behind MSR is that every substance reflects the sun's energy or electromagnetic radiation in different ways. The radiometer contains interference filters that measure incoming sunlight (incident radiation) at the same time reflectance from the forage canopy is being recorded. Other tools, including the Robel pole and disk meter, have been used to estimate standing forage biomass of pastures by estimating canopy height and/or forage density.

Modeling the relationship between reflectance data, visual obstruction measurements, disk meter measurements, and biomass is a complex task. Regression analysis has been used as a modeling tool; however, it lacks the ability to establish rela-

tionships with complex data that deviate from linearity. Alternatively, the development of commercially available neural network software has enhanced our ability to create more dynamic models to predict standing forage biomass.

The practicality of a given procedure will be a function of expense, ease of use, and accuracy of prediction. This experiment was designed to evaluate the accuracy of predicting clipped forage mass from continuously grazed fescue paddocks with multispectral radiometry, Robel pole, or disk meter techniques and to compare the predictive ability of multiple regression versus neural networks in estimating standing forage biomass from MSR data.

Procedures

Study Area

Eight 0.76-ha (1.9-ac), Kentucky 31 tall fescue (*Festuca arundinacea*) paddocks were used for a season-long grazing trial during 2000. Four paddocks, selected at random, were interseeded with red clover at a rate of 5.6 kg/ha (5.0 lb/ac) during March 2000, then fertilized with 21 kg K/ha (19 lb K/ac) and 2 kg B/ha (1.8 lb B/ac). The remaining four paddocks were fertilized with 56 kg N/ha (50 lb N/ac). Paddocks were grazed continuously from April until October at an average stocking rate of 970 kg/ha (866 lb/ac).

Data Collection

Standing forage biomass was measured every 28 days from April through October. In each of the eight paddocks, six plots were selected at each collection date to represent a wide range in biomass. Each plot was scanned with an aerial-view MSR from 2 meters above the ground between 0930 and 1800 hours. With each scan, the following information was recorded: Julian date, time, temperature, sun angle, solar irradiation, plot number, and spectral reflectance at 13 wavelengths: 460, 485, 510, 559, 560, 610, 660, 661, 710, 760, 810, 830, and 1650 nm.

After scans were taken with the MSR, the center of the plot was marked by dropping a flag from the center of the radiometer. Data were then collected using the Robel pole and disk meter. The Robel pole was placed in the center of the plot, and four readings were taken at equidistant points around the pole at a distance of 4 meters and height of 1 meter. Then the disk meter was placed at the center of the plot and displacement of the disk by the forage canopy was recorded (cm). Finally, a 0.25-square-meter quadrat and hand shears were used to collect the vegetation from the plot. The forage was cut at approximately 2 cm above soil level. Within each collection period, 10 clipped samples were selected to determine botanical composition by manual separation into tall fescue, red clover, other grasses, weeds, and senescent material. Clipped samples were then dried at 55°C for 96 hours.

During processing of the MSR data, a leak test confirmed deterioration of the 610 and 810 nm filters. Therefore, information from these wavelengths was removed from the statistical analysis along with data from the 760 nm wavelength because of an equipment error during May collections. The loss of the 610 and 810 nm reflectance values likely limited the predictive value of our data because other studies have shown these wavelengths to be highly correlated to standing forage biomass.

Statistical Analysis

Predictive ability of reflectance data from the MSR was evaluated using two different methods:

- multiple regression and
- a neural network.

Data from each month were combined into one data set. The data set was randomly divided into a calibration set (60% of the data) and a validation set (40% of the data). The calibration set was used to construct multiple regression models using the stepwise procedure of SAS and neural network models using Braincel software. All models were tested using the validation set by regressing observed values against predicted values.

Separate, simple linear regressions of the Robel pole and disk meter results against standing forage biomass were used to determine the accuracy of these tools. Here, we used the same calibration and validation data sets as previously described.

Results and Discussion

In the stepwise procedure, reflectance at 1,650, 660, 661, 507, and 485 nm wavelengths and solar irradiation were significant at the P = 0.15 level and were, therefore, included in the multiple regression model. This model accounted for 46% of the variation in standing forage biomass across the growing season:

Standing forage biomass (kg/ha)=	3,477.4		
	+	3.6	(solar irradiation)
	+	632.2	(485 nm)
	-	587.7	(507 nm)
	-	2,307.3	(660 nm)
	+	1,942.0	(661 nm)
	+	25.8	(1,650 nm)

The fully trained neural network accounted for 59% of the variation in standing forage biomass across the growing season. The variables of greatest importance included reflectance values at 1,650, 710, 507, and 460 nm and sun angle. Variables of the lowest importance included reflectance values at 559 and 830 nm and Julian date. Overall, predictive ability of the trained neural network (R^2 = 0.59) was greater than for the multiple regression model (R^2 = 0.46; Figures 1 and 2). Greater accuracy by the neural network may be due to its greater tolerance for error in highly complex data sets. Even with the loss of the 610 and 810 nm data, the neural network was able to explain approximately 60% of the variation in standing forage biomass. Research with other forage types has indicated that reflectance at 610 and 810 nm is highly correlated with standing forage biomass. Thus, we believe the potential for MSR is underestimated in the present study.

Regression equations for both the Robel pole (R^2 = 0.44; Figure 3) and disk meter (R^2 = 0.44; Figure 4) accounted for little variation in standing forage biomass across the season. Results obtained from the Robel pole and disk meter were lower than expected. However, these results may be attributed to sam-

pling techniques and forage characteristics. The major objective of this experiment was to acquire a broad range of forage biomass samples in order to calibrate all forage measuring devices. In our study, forage samples were collected across seven months, which allowed canopy variation to influence biomass measurements. Changes in physical structure of grass (i.e., development of stems) has a large influence on visual obstruction and disk meter measurements. Increased maturation of grass also increased the occurrence of lodged grass, which leads to poor results at higher dry matter yields.

This experiment indicates that developing alternative methods to estimate standing forage biomass is complex. Even in the absence of reflectance values at key wavelengths, the predictive ability of MSR, combined with the large sampling size made possible by its speed, suggests that it could be very useful for prediction of biomass in fescue pastures. However, when developing new methods to estimate biomass, factors such as forage type, season, physical structure of the forage, and grazing method must be taken into consideration. Furthermore, extensive site-specific calibrations for each measurement device must be conducted before these tools can be considered practical for determination of standing forage biomass.

Summary

Three measuring devices (multispectral radiometer—MSR, Robel pole, and disk meter) were used to measure standing forage biomass of eight 0.76-ha (1.9-ac), tall fescue-based paddocks under continuous grazing (stocking rate = 970 kg/ha; 866 lb/ac). Data were collected every 28 days from April through October 2000. Six plots from each paddock were selected. Each plot was first scanned using MSR, then Robel pole and disk meter readings were taken from the center of the plot. Finally, forage was clipped within a 0.25-square-meter quadrat to determine standing forage biomass.

Multiple regression and neural network models were constructed for the MSR data. Simple regression models were constructed for the Robel pole and disk meter data. Multiple regression models accounted for little of the variation ($R^2 = 0.46$) in standing forage biomass; however, the neural network models accounted for 59% of the variation in standing forage biomass. Simple regression models for the Robel pole ($R^2 = 0.44$) and disk meter ($R^2 = 0.44$) resulted in poorest estimation of standing forage biomass across seasons. Multispectral radiometry offers potential for generating estimates of standing forage biomass, especially when combined with neural network analysis.

Figure 1. Prediction of standing forage biomass from reflectance data using multiple regression models.

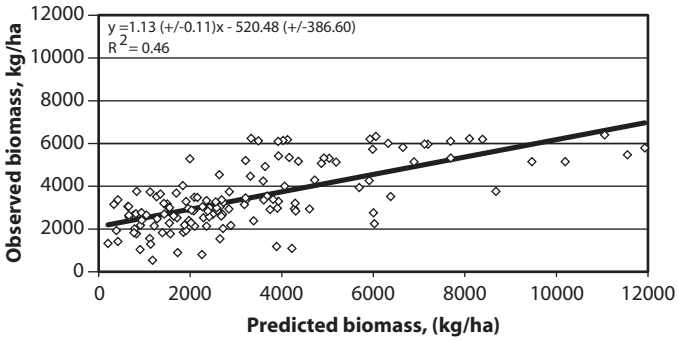


Figure 2. Prediction of standing forage biomass from reflectance data using neural network models.

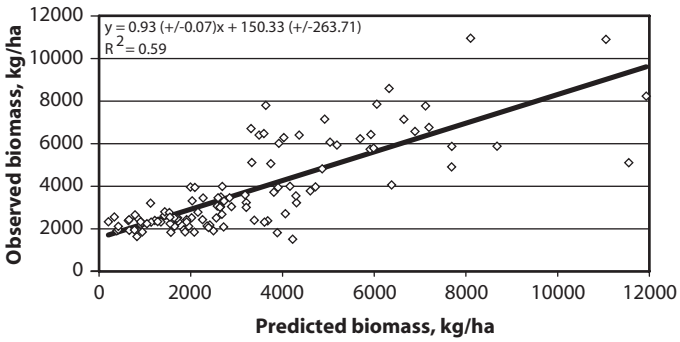


Figure 3. Prediction of standing forage biomass using a Robel pole.

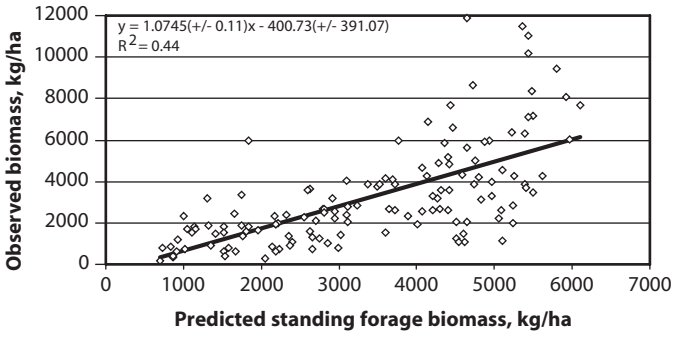
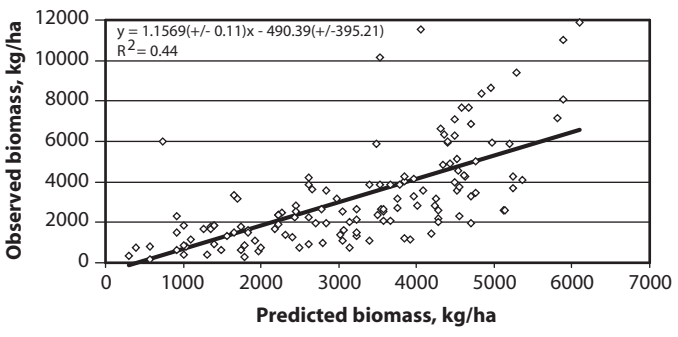


Figure 4. Prediction of standing forage biomass using a disk meter.



The Effects of Procedural Modifications on Alkane Recovery in Forage and Fecal Samples

C.L. Schultz, E.S. Vanzant, D. Harmon, and L. Driedger

Introduction

Indigestible markers are important tools for estimating intake and digestibility of forages consumed by grazing animals. As natural components of plant waxes, n-alkanes have many characteristics that make them attractive for use as indigestible markers. However, procedures for analyzing n-alkanes are somewhat complex, and these procedures must be validated before they can be conducted routinely with a given forage type and within a given laboratory. One factor that can affect recovery of alkanes from a sample is the size of the sample used, relative to the volume of solvent used to extract the alkanes. In these experiments, we examined the influence of sample size on the ability to measure various n-alkanes in fescue and fecal samples, and on the ability to recover known amounts of various alkanes added to the samples prior to extraction.

Procedures

Previously collected forage and fecal samples were used. The forage sample was from a moderate-quality fescue hay. The fecal sample was obtained from a steer grazing stockpiled fescue, and dosed with the even-chain n-alkane, C_{32}. Samples were dried at 55°C in a forced-air oven and ground through a 1-mm screen in a Wiley mill. To determine effects of sample size on measurement of sample alkane concentrations and recovery of spiked alkanes, forage (0.5, 0.75, 1.0, and 1.5 g) and fecal (0.5, 1.0, and 1.5 g) samples were tested using a previously described procedure. The original procedure utilized 1.5 g of forage sample with the same quantities of solvents as described below, or 0.5 g of fecal samples with half of the solvent quantity as used for forage samples. To calculate recovery of spiked alkanes, one set of samples (n = 2) within each sample size received 300 µL of a stock solution containing 1 mg/mL of each of the alkanes C_{21} through C_{36}, excluding C_{24}. A second set of samples received no stock solution and was used to determine n-alkane concentration (n = 2). For calibration purposes, C_{24} was used as an internal standard and added to all samples at a concentration of 0.15 mg/mL. Reagent amounts were similar for all sample types and sample sizes. Samples were digested in 14.0 mL 1 M KOH for 4.5 hours at 90°C followed by the addition of water (4 mL) and heptane (14 mL). Heptane used in this step contained the internal standard. Samples were placed on a bench-top shaker for 30 minutes and then centrifuged for 15 minutes at 5,000 x g. Vigorous shaking was incorporated into the procedure due to relatively low recovery (75%) of alkanes in an earlier validation procedure.

Following centrifugation, the non-aqueous liquid layer was removed and placed into a separate 50-mL tube, and the liquid/liquid extraction was repeated for the removal of a second liquid layer. Samples were then evaporated using filtered air, reconstituted with 3.5 mL heptane (containing no internal standard), and poured over silica gel columns. Columns were washed with an additional 10.5 mL heptane. Samples were evaporated again, reconstituted with 0.4 mL heptane, and placed in glass vials for analysis of alkane concentrations by gas chromatography. We used an HP 6890 gas chromatograph with FID and fitted with a 2-mm i.d. column, packed with Supelco 3% SP-2100. Nitrogen (carrier gas) flow was 20 mL/minute, and the oven temperature was set to increase from 175°C to 300°C at a rate of 8°C/minute.

Data from forage and fecal samples were analyzed separately. Using a model for a completely randomized design, sample size, alkane, and the sample size by alkane interaction effects on concentration and recovery of n-alkanes were analyzed using the GLM procedure of SAS. Linear, quadratic, and cubic effects of sample size were determined using orthogonal contrasts.

Results and Discussion

Average concentrations for C_{25} through C_{35} n-alkanes in fescue and feces are given in Figures 1 and 2. We had difficulties obtaining data for alkanes shorter than C_{25} and for C_{34}. Thus, data for these alkanes were excluded from the analyses. For fescue samples, there was an interaction ($P < 0.01$) between alkane chain length and sample size. However, with the exception of C_{25}, C_{28}, and C_{31}, which had linear ($P \leq 0.03$) sample size effects, increasing sample size resulted in cubic ($P \leq 0.07$) effects on measured alkane concentrations. In general, increasing sample size resulted in decreased concentrations of measured alkanes. The cubic response was indicative of a greater decrease in measured concentration between the 0.75 g and 1.0 g samples than between other sample sizes.

With alkane concentrations in feces (Figure 2), no interaction was detected ($P = 0.52$) between alkane chain length and sample size. The effect of sample size was described by a quadratic curve ($P < 0.01$), in which measured alkane concentrations were greater for 0.5 g and 1.5 g samples than for the 1.0 g sample size. Decreasing alkane concentration measurements with increasing sample size could be explained by saturation of extraction solvents as sample size increased. Likewise, increasing alkane concentration measurements with increasing sample size could be rationalized by the presence of interfering compounds that would be present in greater amounts at higher sample sizes. However, the fact that we detected the lowest fecal alkane concentrations with intermediate sample sizes remains an anomaly.

Alkanes with an odd chain length had the highest concentrations in both fescue and fecal samples because they exist naturally within the plant cuticle. Additionally, the dosed C_{32} was detected in the fecal sample. Concentrations of the various alkanes displayed in Figure 1 are in close agreement with values reported for other cool-season grasses. The relatively large concentrations of C_{31} and C_{33} suggest that these alkanes may be useful as markers with fescue-based diets.

Recoveries of alkanes added to fescue and fecal samples (spiked samples) are presented in Figures 3 and 4. No interaction was detected ($P = 0.36$) between alkane chain length and

Figure 1. Concentration of n-alkanes measured in fescue hay using varying sample sizes. Alkane chain length x sample size interaction (P < 0.01).

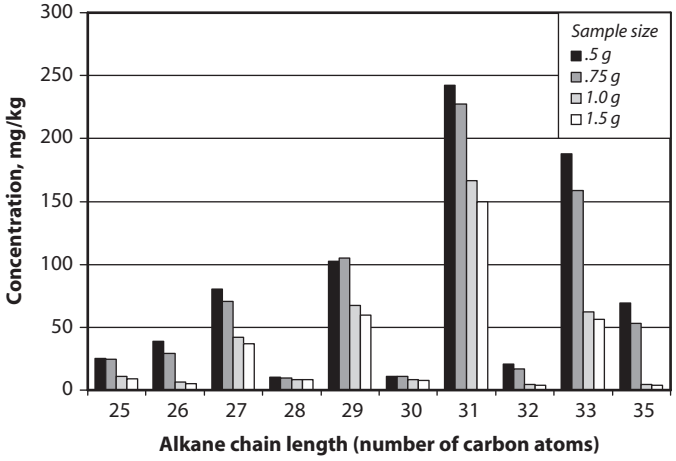
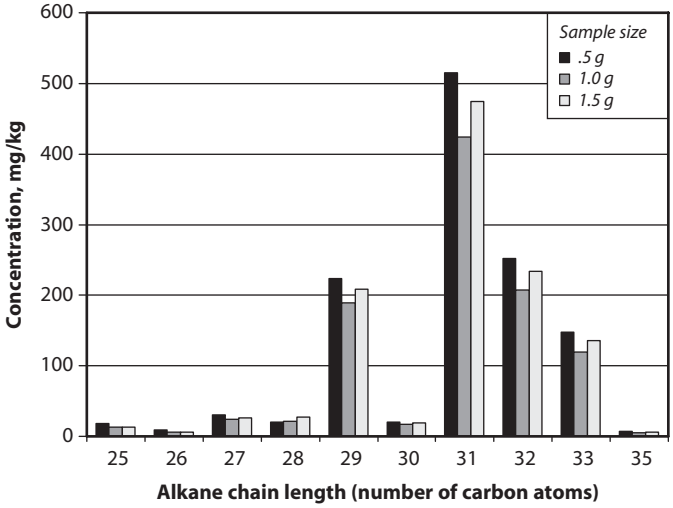


Figure 2. Concentration of n-alkanes measured in feces using varying sample sizes. Alkane chain length x sample size interaction (P = 0.52); Alkane chain length effect (P < 0.01); Sample size effect (Quadratic; P < 0.01).



sample size, indicating that effects of sample size were fairly consistent (cubic; P < 0.01) for all alkanes. For all alkanes, recoveries were greater with 1.0 g sample size (average recovery = 98.5%) than with any of the other sample sizes evaluated. Recoveries of spiked alkanes was fairly similar between the 0.5 g (average recovery = 90.4%) and 0.75 g (average recovery = 90.9%) sample sizes, and lowest with 1.5 g (87.1%). Data suggest that a sample size of 1.5 g may be excessive. Although the recoveries with the 1.0 g sample size were closest to 100% on average, the increase in recovery with increasing sample size from 0.75 g to 1.0 g remains unexplained. Additionally, variation in recoveries was greater with the 1.0 g sample than with other sample sizes.

With spiked fecal samples, recoveries were not affected (P > 0.99) by an alkane x sample size interaction, and recoveries of all alkanes were represented by a quadratic (P = 0.02) relationship to sample size. However, the nature of this relationship was opposite that seen with fescue samples in that recoveries were least with 1.0 g (average recovery = 89.8%), compared with either 0.5 g (average recovery = 96.8%), or 1.5 g (average recovery = 94.8%) sample sizes. Again, this response was unexpected and cannot be explained with the data from this experiment. On average, recoveries with spiked fecal samples were closer to 100% than recoveries with spiked fescue samples. This suggests that some component present in

Figure 3. Recoveries of spiked n-alkanes from forage samples using varying sample sizes. Alkane chain length x sample size interaction (P = 0.36); Alkane chain length effect (P < 0.01); Sample size effect (Cubic; P < 0.01).

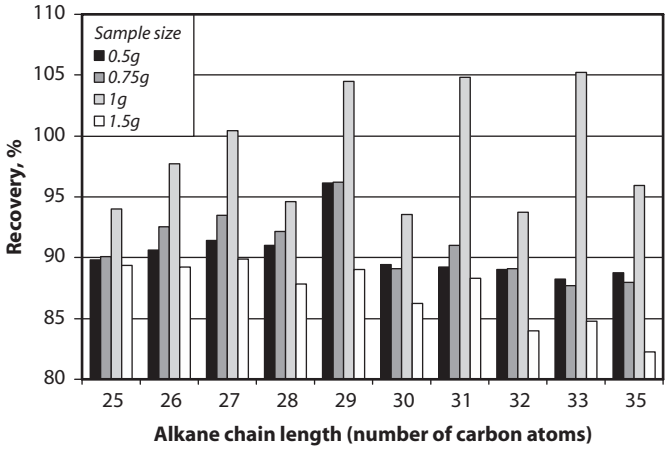
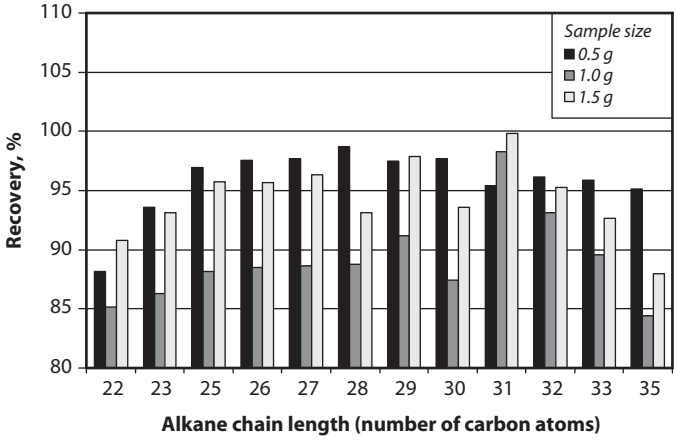


Figure 4. Recoveries of spiked n-alkanes from feces using varying sample sizes. Alkane chain length x sample size interaction (P > 0.99); Alkane chain length effect (P = 0.97); Sample size effect (Quadratic; P = 0.03).



fescue hay, but not in feces, may interfere with quantitative isolation and/or measurement of alkanes.

Changing the amount of sample extracted with a given quantity of solvent resulted in differences in measured concentrations of endogenous alkanes and in recovery of added alkanes from both fescue and fecal samples. However, changes were not consistent with increasing sample size. Additional work using a wider range in sample sizes is being conducted to more clearly define the effect of sample size on our ability to extract and quantify alkanes from forage and fecal samples.

Summary

Indigestible markers are important tools for estimating intake and digestibility of forages consumed by grazing animals. Natural components of plant waxes, n-alkanes have many characteristics that make them attractive for use as indigestible markers. This experiment demonstrated that our ability to accurately measure alkanes in samples of forage and feces may be related to the sample size used in the procedure.

We added known amounts of alkanes varying in chain length from C_{21} to C_{36} to fescue and fecal samples of various sizes (0.5, 0.75, 1.0, and 1.5 g). Using a previously described extraction and analysis procedure, we compared measured alkane concentrations to the known quantity of each added alkane and calculated recoveries as a percentage of the amount spiked.

Measurements of alkane concentrations in unadulterated samples and recoveries of spiked alkanes were influenced by sample size in both fescue and feces. However, there were no consistent trends in recoveries with changes in sample size. Additional studies are necessary to delineate the most appropriate techniques to use for alkane determination.

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