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Influence of Levels of Fat Supplementation on Bile Flow and Fatty Acid Digestion in Cattle

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Abstract: Three Jersey x Holstein steers (215 " 4.8 kg) with cannulas in the rumen and proximal duodenum were used in a 3 x 3 Latin squares design experiment to evaluate the level of fat supplementation (0, 4, and, 8% yellow grease) on characteristics of duodenal chyme, bile production, and digestion of fatty acids. Dry matter intake was restricted to 2.15% of BW. Characteristics of bile obtained during evisceration of 18 beef carcasses were as follows: pH = 7.48 ± 0.29; total solids = 8.32% ± 0.66; density, 1.012 ± 0.02 g/mL and total lipids, 1468 ± 82 mg/dL. Increasing level of fat supplementation decreased (linear effect, P = 0.07) postruminal fatty acid digestion, due primarily to decreased absorption of saturated fatty acids. The estimated NE_m (Mcal/kg) of yellow grease averaged 5.87 and 5.46 for the 4, and 8% of level supplementation, respectively. There were no treatment effects ($P>0.20$) on pH and density of duodenal chyme. Although, pH was lower (2.34 vs 3.81, $P<0.01$), and density was greater (1.3% $P<0.01$) for proximal duodenum than for distal duodenum chyme. Bile production averaged 31.9 ± 0.35 mL/kg BW. Increasing level of fat intake increased (linear effect, P = 0.07) bile production. Increasing the level of supplemental fat from 4 to 8% increased bile production and duodenal lipid flow by 10.3 and 35.7%, respectively. Thus, with increasing level of fat supplementation, the bile:lipid ratio (mL of bile/g of lipid in duodenum) of distal duodenal chyme decreased (linear effect, $P<0.01$) from 23.4 to 16.7. The bile:lipid ratio of distal duodenal chyme explained 69% of the variation in intestinal fatty acid digestion. We conclude that the decrease in NE value of supplemental fat with increasing level of fat intake is largely due to disproportionate increases in bile production, and hence, decreased bile:lipid ratios.

Key words: Fat level, Fatty acid, Cattle, Bile flow, Digestion.

Introduction

Current standards (NRC, 1996) for the NE_m and NE_g values of supplemental fats are 6.00 and 4.50 Mcal/kg. Estimates based on these values are consistent with empirically derived measures when total fatty acid intake did not exceed 0.86 g/kg of BW (Zinn, 1994). When fatty acid intake exceeded 0.86 g/kg of BW, the NE value of fat declined (Zinn and Plascencia, 2002). This decline has been largely attributable to decreased postruminal fatty acid digestion (Zinn, 1994), mainly stearic and palmitic acids (Enjalbert *et al.*, 2000 and Plascencia *et al.*, 2003). Bouchart (1993) suggested that decreased fatty acid digestion might be due to insufficient bile production. The flow of digesta into the duodenum stimulates bile secretion rates (Harrison, 1962). However, the influence of fat intake on bile secretion has not been assessed in ruminants. Early reports (Harrison and Hill, 1960 and Harrison, 1962) indicated wide discrepancies (0.6 to 1.45 mL/h/kg BW) in estimates of bile production in sheep. The basis for this variation is not certain. Bile flow was determined using permanent cannulation of the common bile duct of sheep fed forage-based diets. Collected bile was not returned to the duodenal , interfering with normal recycling (enterohepatic circulation; Merchen, 1988). As an alternative to cannulation of the bile duct, net bile production may also be determined as the difference in bile flow between intestinal cannulas located proximal and distal to the bile duct. Lipid absorption along this first segment of the small intestine (about 60 cm) is negligible due to low pH (1.8-2.7, Leat and Harrison, 1969), and high rate of passage (Bueno *et al.*, 1975). The objective of present study was to directly assess the relationship between bile production and intestinal fatty acid digestion in cattle fed high-grain finishing diets.

Materials and Methods

Three Jersey x Holstein steers (215 " 4.8 kg) with cannulas in proximal (6 cm of pyloric sphincter; Zinn and Plascencia, 1993) and distal duodenum (10 cm posterior to the bile duct) were used in a 3 x 3 Latin square design to evaluate the influence of level of fat supplementation (0, 4 and 8%) on characteristics of bile flow and fat utilization in cattle fed finishing diets. Composition of experimental diets is shown in Table 1. Composition of the yellow grease (**YG**) used in this study (Table 2) was similar to the standards set by American Fats and Oils Association (AFOA, 1988), and to measures for YG used in other experiments conducted at this center (Zinn,

1988, 1989b, 1992). Supplemental fat was added to the diet during the next to the last step in the batch mixing process, just prior to adding molasses.

Eighteen gall bladders were obtained during postmortem evisceration of beef carcasses at a commercial slaughter plant. Bile from excised gall bladders was quantitatively transferred into individual plastic jars (500 mL). Sample-jars were then placed on ice and promptly transported to the laboratory for immediate analyses.

Steers were individually maintained in concrete slotted-floor pens (3.9 m^2) with access to water at all times. Dry matter intake was restricted to 2.15% of BW (4.6 ± 0.056 kg/d). Diets were fed in equal portions at 0800 and 2000 daily. Experimental periods consisted of a 10-d diet adjustment period followed by a 4-d collection period. During the collection period duodenal and fecal samples were taken from all steers, twice daily as follows: d 1, 0750 and 1350 h; d 2, 0900 and 1500 h; d 3, 1050 and 1650 h; and d 4, 1200 and 1800 h. Individual samples consisted of approximately 750 mL proximal duodenal chyme, 750 mL from distal duodenal chyme, and 200 g (wet basis) fecal material. Samples from each steer and within each collection period were composited for analysis. Feed and digesta samples were subjected to all or part of the following analysis: DM (oven drying at 105 °C until no further weight loss); ash (AOAC, 1986); fatty acids (Sukhija and Palmquist, 1988); lipid (acidified chloroform-methanol extraction; Zinn, 1994); pH (pHmeter-Orion Mod 2345); density (w/v); and chromic oxide (Hill and Anderson, 1958). Lipid content and physical characteristics of bile used as a reference to calculate bile flow were determined as follows: density (w/v); total solids (oven drying at 65°C until no further weight loss); pH (pHmeter-Orion Mod 2345); and total lipid (colorimetric method; TECO diagnostics, Anaheim, CA). Composition of supplemental yellow grease was analyzed according to AOCS (1978) procedures as follows: moisture (method Ca 2a-45), impurities (method Ca 3-46), unsaponifiables (method Ca 6a-40), iodine value (method Tg 1a-64). Bile flow was calculated as the difference in lipid content in duodenal chyme divided by lipid concentration the bile reference:

Bile flow, L/d = (lipid in distal duodenum-lipid in proximal duodenum)/lipid concentration of reference bile, g/L

Data for bile flow and digestion of lipid and fatty acids were analyzed using a 3 x 3 Latin square design (Hicks, 1973). Treatment effects were tested using the orthogonal polynomials. Characteristics of duodenal chyme were analyzed using a 3 x 3 Latin square design with repeated measures, as outlined by Hicks (1973).

Results and Discussion

Characteristics of reference bile obtained during evisceration of 18 beef carcasses were as follows: pH = 7.48 ± 0.29; total solids = 8.32% ± 0.66; density, 1.012 ± 0.02 g/mL and total lipids, 1468 ± 82 mg/dL. These results are close in agreement with those reported by others (Ruckebusch *et al.*, 1991; Moore and Christie, 1984; Merchen, 1988). There were no treatment effects ($P>0.20$) on density and pH of duodenal chyme. Although, density was greater (1.3% $P<0.01$), and pH was lower (2.34 vs 3.81, $P<0.01$) for proximal duodenal chyme than for distal duodenal chyme. Increased pH at the distal duodenum reflects bile and pancreatic secretion. Differences in pH between the proximal and distal segments of the duodenum are consistent with previous studies (Moore and Christie, 1984; Christiansen and Webb, 1990). Although very little has been reported with respect to chyme density, it is reasonable to expect that density would be lower in the distal duodenum due to dilution with bulky bile salts.

Bile production is shown in Table 3. Across treatments, bile secretion averaged 32 mL/kg BW, in good agreement previous studies (32.4 mL/kg BW, Studzinsky and Bobowiec, 1979; 34.8 mL/kg BW, Harrison, 1962; 26.8 mg/kg BW, Bobowiek and Kosior-Korzecka, 1999). Fat supplementation increased (linear effect, P = .07) bile secretion. However, bile secretion per g of lipid flow to the small intestine decreased (linear effect, P < .01) with increasing level of fat supplementation.

In cattle, intestinal fat digestion is a predictable function of level of fat intake (Palmquist, 1991; Zinn, 1994 and Plascencia *et al.*, 2003). Decreases is fat digestion with increasing level of fat intake are largely attributable to decreased digestibility of saturated fatty acids (Wu *et al.*, 1991; Pantoja *et al.*, 1996; Plascencia *et al.*, 1999). Lipids are highly insoluble in water, their absorption from the small intestine is dependent on emulsification and the formation of micelles mediated by bile acids. Unsaturated fatty acids are physically much more bulky and less viscous than saturated fatty acids. Consequently, the critical bile concentration necessary for micellar formation is less than that needed for saturated fatty acids (Freeman, 1984). Although fat supplementation evokes increased bile flow, the magnitude of the increase is disproportionately low compared with the increase in saturated fatty acid flow to the small intestine as a result of ruminal biohydrogenation. In the present study, the bile:lipid ratio explained 69% of variation in intestinal fatty acid digestion (Fig. 1).

Treatment effects on flow and digestion of fatty acids are shown in Table 4. Because DMI was constant across treatments (4.6 kg/d) intakes of individual fatty acids varied according to the content of the diets. Flow of fatty acids to the small intestine was 103, 107, and 107 % of intake for the 0, 4, and 8% levels of supplemental fat, respectively, reflecting de novo microbial fatty acid synthesis (Klusmeyer and Clark, 1991; Pantoja *et al.*, 1996; Ramirez and Zinn, 2000).

Table 1: Ingredient and nutrient composition of experimental diets

	Fatty acids intake, g/kg BW		
Item	0.51	1.07	1.72
Ingredient, g/kg (DM basis)			
Alfalfa hay	60	60	60
Sorghum sudan hay	60	60	60
Steam-flaked corn	781	741	705
Yellow grease	---	40	80
Urea	7	8	9
Molasses	63	62	61
Limestone	13	13	13
Magnesium oxide	2	2	2
Trace mineral salt[a]	4	4	4
Chrornic oxide[b]	4	4	4
Nutrient composition (DM basis)			
NE, Mcal/kg[c]			
Maintenance	2.13	2.27	2.41
Gain	1.47	1.59	1.72
Crude protein, %	11.6	11.50	11.41
Ether extract, %	3.6	7.4	11.2
Calcium, %	0.65	0.67	0.68
Phosphorus, %	0.30	0.29	0.29

[a]Trace mineral salt contained: $CoSO_4$, 0.068%; $CuSO_4$, 1.04%; $FeSO_4$, 3.57%, ZnO, 1.24%; $MnSO_4$, 1.07%; KI, 0.052% and NaCl, 92.96% [b]Chromic oxide was added as a digesta marker
[c]Based on tabular values for individual feed ingredients (NRC, 1996)

Table 2: Composition of supplemental yellow grease

Item	Yellow grease
Fatty acid, %	
C16:0	15.55
C18:0	8.80
C18:1	48.88
C18.2	15.13
Others	
Iodine value, g iodine/100 g fat[a]	82.77
Moisture, %	0.60
Impurities, %	0.50
Unsaponifiable matter, %	0.62

[a]Measure of the degree of saturation of fatty acids

Table 3: Calculated bile flow in steers (215kg) fed diets with 3 level of supplemental yellow grease

	Supplemental yelow grease, %			
Item	0	4	8	SEM
Steers	3	3	3	
Lipids, g/d				
Intake	128.4	253.8	405.9	
Flow to proximal duodenum[a]	156.6	279.4	434.7	3.9
Flow to distal duodenum[a]	251.7	375.0	541.2	3.3
Difference	95.1	95.6	106.5	
Bile flow				
mL/d[b]	6483	6510	7254	149
mL/[kg BW][b]	30.4	31.4	34.2	0.8
mL/[kg BW h][b]	1.26	1.29	1.42	0.03
mL bile/lipid flow to duodenum[a]	41.2	23.4	16.7	1.07

[a]Fat supplementation linear effect, $P<0.01$ [b]Fat supplementation linear effect linear effect, $P<0.10$

Table 4: Main effects of leve of fat supplementation of fatty acid digestion in Holstein steers

	Supplemental yellow grease, %			
Item	0	4	8	SEM
Steers	3	3	3	
Intake, g/d	4586	4616	4613	
Fatty acid intake, g/d				
C16:0	25.3	88.5	104.5	
C18:0	4.2	46.2	56.7	
C18:1	36.7	164.9	240.3	
C18:2	54.6	54.2	39.4	
Total	109.6	228.9	369	
Fatty acid intake, g/kg BW	0.51	1.07	1.72	
Flow to duodenum, g/d				
C16:0[a]	19.45	45.0	75.32	4.7
C18:0[a]	52.8	117.6	206.5	9.7
C18:0[b]	24.7	63.0	129.9	17.4
C18:0[a]	12.2	15.3	18.3	0.4
Total fatty acid[a]	112.8	245.4	394.0	9.4
Fecal excretion, g/d				
C16:0[c]	3.96	8.56	20.57	2.3
C18:0[c]	10.5	31.0	67.8	0.6
C18:1[b]	2.9	9.3	23.1	3.6
C18:2	1.2	1.7	3.0	0.4
Total fatty acid[a]	22.4	58.4	115.9	3.6
Postruminal digestion, %				
C16:0	79.5	79.9	72.6	4.3
C18:0	79.2	72.2	67.7	4.7
C18:1	88.0	85.5	82.0	2.1
C18:2	90.2	88.5	81.9	2.0
Saturated fatty acids	81.5	75.9	63.9	5.1
Unsturated fatty acids[c]	88.7	85.5	81.7	1.1
Total fatty acid[b]	82.7	78.6	72.6	1.9

[a]Fat supplementation linear effect, $P<0.01$ [b]Fat supplementation linear effect linear effect, $P<0.10$
[c]Fat supplementation linear effect linear effect, $P<0.05$

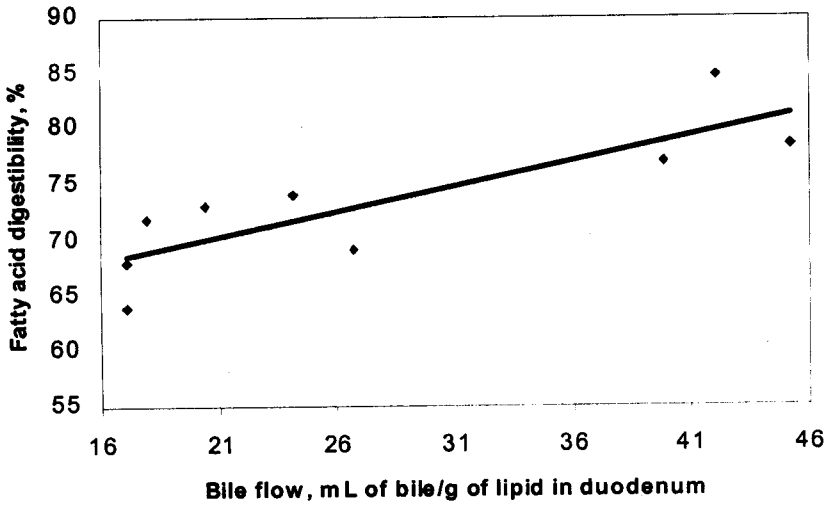

Fig. 1: Influence of bile flow on intestinal fatty acid giestion

Postruminal fatty acid digestion decreased (linear component, P = 0.07) with increasing level of fat supplementation, averaging 80.2, 78.6 and 72.63% for the 0, 4 and 8% level of fat supplementation, respectively. As mentioned previously, has been previously demonstrated (Zinn, 1994) postruminal digestion of supplemental fatty acids is largely a function of total fatty acid intake. Plascencia *et al.* (2003) observed a close relationship (R^2 = 0.89%) between the total fatty acid intake (FAI, g/kg BW) and postruminal fatty acid digestion: fatty acid digestion (%) = 87.560 – 8.591FAI. Accordingly, observed postruminal fatty acid digestion in the present study was 1.00 and 0.99% of expected for the 4 and 8% levels of supplemental fat, respectively.

Post ruminal digestion of saturated and unsaturated fatty acids averaged 73.8 and 85.3%, respectively. The greater digestibility of unsaturated fatty acids is consistent with previous studies (Enjalbert *et al.*, 2000;Zinn *et al.*, 2000 and Avila *et al.*,2000). Digestibility of both saturated (linear effect, P = 0.13) and unsaturated fatty acids (linear effect, P < 0.05) declined with increasing fat intake. Nevertheless, the slope of the decline was 243% greater (2.14 vs 0.88% per unit increase in supplemental fat) for the saturated fatty acids. Furthermore, the decline in postruminal digestion with increasing level of fat intake was 109% greater for C18:0 than for C16:0, consistent with the concept that intestinal digestion of saturated fatty acids decreases with increasing chain length (Steele and Moore, 1968; Zinn, 1989b). At low to medium levels of fat supplementation (less than 5% supplemental fat), postruminal digestibility of C18:0 has been consistently 5% less than that of C16:0 (Bauchart, 1993). However, these differences become greater at higher levels of fatty acid intake (greater than 5% supplemental fat and Plascencia *et al.*, 2003).

Because C18:0 is the predominant fatty acid entering the small intestine (comprising 48 to 52% of total fatty acid flow), the decrease in digestion of C18:0 with increasing levels of fat intake explained 85% (P<0.01) of the variation in total intestinal fatty acid digestion. This result is consistent with previous studies conducted at this Center, where similar fat source and levels of supplementation were compared (Plascencia *et al.*, 1999, 2003 and Zinn *et al.*, 2000). However, a delimitation of metabolism studies of this nature is that it doesn't take into consideration the potential for biohydrogenation of unsaturated fatty acids in the lower intestinal tract. To the extent that some C18:1 entering the small intestine was hydrogenated to C18:0 before excretion in the feces, intestinal digestion of C18:1 and C18:0 would be overestimated and underestimated, respectively.

Given that one gram of intestinally digestible fat (IDF) has a ME value of 9 Kcal (100% of its physiological fuel value); and the partial efficiency of utilization of ME from dietary fat for BW gain is 67% (Czerkawsky *et al.*, 1966; Garrett, 1980 and Zinn, 1994), the NE_g value of dietary fat may be calculated as 6.03 Kcal/g IDF. Accordingly, the NE_g values for the yellow grease used in this study are 4.74, and 4.38 Mcal/kg for the 4, and 8% of level supplementation, respectively. Corresponding NE_m values are 5.87, and 5.46 Mcal/kg, respectively [where NE_m = (NE_g + 0.41)/0.877; derived from NRC, 1984]. Thus, NE values obtained for yellow grease supplemented at the rate of 4, and 8% of dietary DM (1.07, and 1.72 g fatty acid intake/kg BW) were 98, and 91% of the tabular value (NRC, 1996).

Conclusion

Bile production explains 69% of variation in fatty acid intestinal digestibility. Depressions in fatty acid digestion with increasing level of intake are due primarily to negative effect on intestinal absorption of stearic acid. Intestinal fatty acid digestion, and the net energy value of supplemental fat is a predictable function of total fatty acid intake.

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