Evaluation of catfish oil as a feedstuff for lactating Holstein cows¹

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ABSTRACT

The objective of this study was to evaluate catfish oil (CFO) as a dietary ingredient for lactating Holstein cows. Twelve multiparous Holstein cows (6 fitted with a rumen cannula and 6 noncannulated cows), arranged in a 3 × 3 Latin square design replicated 4 times, were used to evaluate CFO as a dietary ingredient for lactating Holstein cows. Each period lasted 27 d with the last 13 d used for data collection. Dietary treatments were 0, 1.5, and 3% CFO (dry matter basis). Orally dosing with chromium oxide powder was used as an external inert marker for calculation of apparent dry matter and nutrient digestion coefficients. Ruminal fluid was collected hourly for 8 h after feeding to measure pH and volatile fatty acids. Intake of dry matter increased as intake of CFO increased (23.0, 24.4, and 25.4 kg/d). Production of milk was unchanged by the feeding of CFO (29.0, 29.0, and 29.4 kg/d). Concentrations of milk fat (3.57, 3.60, and 3.48%) and protein (3.21, 3.18, and 3.23%) were unchanged by feeding CFO. Concentrations of plasma glucose (57.8, 55.1, and 56.0 mg/100 mL), urea nitrogen (11.6, 11.0, and 12.0 mg/100 mL), and insulin (0.55, 0.53, and 0.57 ng/mL) were not affected by dietary treatments. Average ruminal fluid pH decreased (6.40, 6.20, and 6.15), as did the molar proportions of acetate (64.5, 64.2, and 63.4%), as dietary concentration of CFO increased. The molar proportions of propionate increased (19.4, 20.0, and 20.4%) as did that of butyrate (12.0, 12.4, and 12.5%) as intake of CFO increased. Ruminal protozoa numbers were unchanged by treatments. Apparent digestibility coefficients of dry matter, crude protein, neutral detergent fiber, and acid detergent fiber were increased by addition of CFO. In situ lag, rate, and extent of corn silage dry matter digestion were not affected by the inclusion of CFO. However, in situ digestion rate of neutral detergent fiber was increased (0.023, 0.024, and 0.029 h⁻¹) with increasing intake of CFO. In a sec-

ond study involving 190 Holstein cows, those fed CFO at 1.8% of dietary dry matter produced 1.2 kg more milk/d than those not fed CFO, along with an increase in milk protein concentration. Catfish oil can be a viable lipid source for dairy cows when fed at up to 3% of the dietary dry matter.

Key words: catfish oil, fat, digestibility

INTRODUCTION

Reductions in DMI and milk production occur when dairy cows are exposed to heat stress conditions. The addition of fats to dairy cow diets during the summer period can be an excellent choice because fats are energy dense, result in less heat increment in the digestive tract, and do not contribute to ruminal acidosis as the over-feeding of starch does (Palmquist and Jenkins, 1980). Fats can be supplied from vegetable or animal sources. The responses of the dairy cow to fat supplementation can depend upon the fatty acid profile of the fat supplement and upon the type of dietary forage (Smith et al., 1993; Ruppert et al., 2003). Several studies reported that supplemental fat increased milk production (DePeters et al., 1987; Chilliard and Doreau, 1997; Keady et al., 2000) but also could depress DMI. The responses were dependent partially upon the fat concentration in the dietary DM.

In the search for fat supplements to use in dairy cow diets, preliminary work in Florida indicated that catfish oil (CFO) mixed with liquid molasses dramatically improved intake of the liquid supplement by beef cows on rangeland (F. M. Pate, University of Florida; personal communication). Catfish oil has not been evaluated as a feedstuff for dairy cows. Approximately 12,000 to 14,000 tons of CFO is generated each year as a byproduct of the commercial catfish meat industry. Catfish offal is separated into solid and liquid fractions by cooking at 129°C. The liquid is centrifuged to separate water from the oil, which is collected, refined, and put into settling tanks before shipment. Because catfish are freshwater fish, their fatty acid profile is different from that of marine fish in that the n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid are in lower concentrations. This is likely because pond-raised freshwater fish are fed a commercial pellet and consume

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Table 1. Composition and quality measures of catfish oil

Measure	Value
Fatty acid, % of fatty acids	
Myristic acid	1.8
Palmitic acid	18.7
Palmitoleic acid	4.9
Stearic acid	5.0
Oleic acid	47.0
Linoleic acid	14.0
Linolenic acid	1.2
Eicosapentaenoic acid	1.1
Docosahexaenoic acid	3.1
Other	3.1
Total fatty acids, %	94.5
Free fatty acids, %	5.7
Iodine value	86
Peroxide value, mEq/kg of oil	2.2
Moisture and volatile matter, %	0.2
Insoluble impurities, %	0.02
Unsaponifiable matter, %	1.0

less algae and plankton, excellent sources of n-3 fatty acids for marine fish (Henderson and Tocher, 1987). Feeding marine fish oil has improved milk production but can decrease DMI (Chilliard and Doreau, 1997; Keady et al., 2000). If CFO can improve feed intake for dairy cows as it did with beef cows, it may prove to be a very effective energy supplement for increasing milk production. The objective of this study was to evaluate CFO as a dietary ingredient for lactating Holstein cows.

MATERIALS AND METHODS

Approval by the Institutional Animal Care and Use Committee of the University of Florida was obtained before initiation of this study.

Experiment 1

Cows and Diets. Twelve multiparous, lactating Holstein cows (6 ruminally fistulated; mean of 195 ± 27 DIM) were arranged in a 3×3 Latin square design replicated 4 times. Two squares were composed of ruminally fistulated cows and 2 squares were composed of nonruminally fistulated cows. The 3 dietary treatments were 0, 1.5, and 3.0% CFO of dietary DM. Unsaturated fats (72%) are predominant in CFO, with oleic acid in greatest concentration (Table 1). Concentration of FFA, moisture, and impurities were low, typical of high-quality fats. As the proportion of CFO increased in the diet, the proportion of whole cottonseed decreased, whereas that of soybean meal increased to maintain isonitrogenous diets (Table 2). Traditionally whole cottonseed is a significant source of fat in many dairy cow diets in the United States. Catfish oil (Protein Products

Inc., Gainesville, GA) was suspended in liquid sugar-cane molasses (20% as-is basis; United States Sugar Corp., Clewiston, FL) and stored in a 6,000-L plastic tank on farm. A second tank contained molasses without CFO. The molasses-CFO blends were mixed with concentrate ingredients in 1-ton batches. Concentrates were mixed with corn silage and alfalfa hay at the time of feeding in a weighing and mixing unit (American Calan, Inc., Northwood, NH) and offered as TMR twice daily at 0930 and 1400 h for ad libitum consumption allowing 10%orts. Water was available continuously in ad libitum amounts.

The experiment was conducted at the University of Florida from August to November and consisted of three 27-d periods. The first 14 d of each period was used to adapt cows to a new diet and the last 13 d used for data collection. Cows were housed in a free-stall, open-sided barn fitted with Calan gates (American Calan Inc.) to allow measurement of feed intake by individual cows. Free-stalls were bedded with sand and cleaned daily. Sufficient free-stalls were available to provide at least 1 free-stall per cow. Fans and misters were operated continuously in the barn for cooling purposes. Temperature and humidity were recorded outside and inside the barn every 15 min throughout the experiment (Onset Computer Corp., Bourne, MA).

Collection of Samples and Analysis. Weights of feed offered and orts were recorded daily for each cow. Representative samples of concentrate mixes, corn silage, and alfalfa hay were collected weekly and composited for each experimental period. Corn silage and alfalfa hay samples were dried at 55°C in a forced-air oven and ground to pass the 1-mm screen of a Wiley mill (A. H. Thomas, Philadelphia, PA) before compositing. Feedstuff samples were analyzed for DM (105°C for 8 h), OM (512°C for 8 h), NDF (Van Soest et al., 1991) using heat-stable α -amylase, ADF (AOAC, 1990), 80% ethanol soluble carbohydrate (Hall et al., 1999), starch (Hall et al., 1999), Kjeldahl N (AOAC, 1990; CP was calculated by multiplying Kjeldahl N $\times$ 6.25), and fat (acid hydrolysis; AOAC, 1990; method 954.02). In addition, mineral composition was determined by Dairy One (Ithaca, NY) using a Thermo Jarrell Ash IRIS Advantage HX inductively coupled plasma radial spectrometer (<http://www.dairyone.com/Forage/Procedures/default.htm>; accessed January 2009).

Cows were milked daily at 0500, 1300, and 2100 h and milk weights were recorded. Milk samples were collected for 2 consecutive milkings on d 16, 17, 23, and 24 of each period for determination of fat, protein, and SCC. Somatic cell scores were generated as described by Norman et al. (2000) for statistical analysis of SCC. Samples were analyzed by Southeast Dairy Labs (McDonough, GA) by infrared technologies (Bentley 2000,

Table 2. Ingredient and chemical composition of experimental diets containing catfish oil (CFO) fed to lactating Holstein cows in summer in experiment 1

Item	0% CFO	1.5% CFO	3.0% CFO
Ingredient			
Corn silage, % of DM	29.9	29.9	29.9
Alfalfa hay, % of DM	11.5	11.5	11.5
Cottonseed hulls, % of DM	4.7	4.7	4.7
Corn meal, % of DM	17.2	17.2	17.0
Soybean meal, % of DM	11.5	13.0	14.4
Citrus pulp, % of DM	5.6	5.6	5.6
Whole cottonseed, % of DM	5.2	2.6	0
Prolak, ¹ % of DM	1.5	1.5	1.5
Liquid molasses, ² % of DM	8.1	4.0	0
Liquid molasses + CFO, ³ % of DM	0	5.3	10.5
Mineral and vitamin mix, ⁴ % of DM	4.4	4.4	4.4
Biophos, ⁵ % of DM	0.3	0.3	0.5
Chemical			
CP, % of DM	18.3	18.3	18.5
NDF, % of DM	30.3	30.9	28.5
ADF, % of DM	18.9	18.7	17.1
Fatty acids, % of DM	2.8	3.4	3.7
Starch, % of DM	23.1	23.3	24.1
Sugars, % of DM	9.3	8.7	9.3
NE _L , Mcal/kg	1.58	1.62	1.65
Ca, % of DM	1.31	1.35	1.34
P, % of DM	0.47	0.47	0.49
Mg, % of DM	0.38	0.40	0.44
K, % of DM	1.79	1.77	2.10
Na, % of DM	0.51	0.49	0.50
S, % of DM	0.34	0.34	0.32
Cu, mg/kg of DM	32	32	29
Fe, mg/kg of DM	244	250	260
Mn, mg/kg of DM	104	110	108
Zn, mg/kg of DM	99	122	119

¹Protein supplement of high RUP concentration; H. J. Baker & Bro., Inc., Stanford, CT.

²United States Sugar Corp., Clewiston, FL.

³CFO supplied by Protein Products, Inc., Gainesville, GA, to United States Sugar Corp. for mixing at 20.0% (as-is basis).

⁴Mineral and vitamin mix contained 26.4% CP, 1.74% fat, 10.15% Ca, 0.90% P, 3.1% Mg, 8.6% Na, 5.1% K, 1.5% S, 4.1% Cl, 2,231 mg of Mn/kg, 1,698 mg of Zn/kg, 339 mg of Fe/kg, 512 mg of Cu/kg, 31 mg of Co/kg, 26 mg of I/kg, 7.9 mg of Se/kg, 67,021 IU of vitamin A/kg, 19,845 IU of vitamin D/kg, and 357 IU of vitamin E/kg (DM basis).

⁵IMC-Agrico, Bannockburn, IL.

Bentley Instruments, Chaska, MN). Two consecutive milk samples were collected on d 23 of each period for fatty acid analysis. These samples were stored at -20°C . Milk fat was extracted by the method of Chilliard et al. (1991). The extracted oil was placed in 15-mL Pyrex, leak-proof, Teflon-lined screw cap tubes, flushed with N, and sent to Clemson University (Clemson, SC) on dry ice for analysis by gas chromatography (Jenkins, 2000). Because of extended exposure of some samples to room temperature, only 25 of 36 cow-period samples were analyzed for fatty acids.

Body weight was monitored by weighing the cows on 2 consecutive days at the beginning and the end of each period before the a.m. milking. Rectal temperatures were measured twice daily at 0430 and 1630 h on d 16, 18, 20, 22, 24, and 26 of each period.

Blood samples (~ 10 mL) were collected from the coccygeal vessels into Vacutainer tubes containing sodium

heparin (Becton, Dickinson and Co., Franklin Lakes, NJ) on d 26 and 27 of each period. Blood was stored on ice for transport and centrifuged at $1,916 \times g$ for 15 min to separate plasma. Plasma was stored at -20°C until analyzed.

A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to measure plasma glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and BUN (Bran and Luebbe Industrial Method 339-01; Marsh et al., 1965). Plasma insulin was analyzed using a double antibody radioimmunoassay procedure as described by Soeldner and Slone (1965) and modified by Malven et al. (1987). Bound radioactivity in tubes was measured using a Packard auto gamma counter (model B-5005). The results were calculated using the log-logit curve fit. Sensitivity of the assay was 0.2 ng/mL and the intra-assay coefficient of variation was 11.8%.

At 1630 h on d 23 and 24 of each period, spot samples of urine were collected and measured for pH (Horiba twin pH meter B-213, Spectrum Technologies, Inc., Plainfield, IL). Urine samples were frozen at -20°C and kept for analysis. To estimate microbial protein synthesis in the rumen, creatinine and allantoin analyses were performed as described by Vagnoni et al. (1997). At the same time as urine collections, feces (80 g) were collected, diluted in 80 mL of distilled water immediately after collection, and measured for pH (pH meter, model 15; Fisher Scientific, Pittsburgh, PA).

Apparent Digestibility. Gelatin capsules containing 10 g of Cr_2O_3 were administered orally via a balling gun twice daily at 0430 and 1630 h on d 15 to 24 of each period. Fecal grab samples were collected before the administration of the capsules on d 20 to 24 of each period. Fecal samples from daily collections were dried at 55°C in a forced air oven and ground to pass the 1-mm screen of a Wiley mill (A. H. Thomas). Samples of dried feces were composited across the 10 sampling times to obtain one fecal sample per cow per period. Feces were analyzed for Cr by atomic absorption spectrophotometry (Williams et al., 1962), DM (105°C for 8 h), OM (512°C for 8 h), NDF (Van Soest et al., 1991) using heat-stable α -amylase, ADF (AOAC, 1990), Kjeldahl N (AOAC, 1990), and fat (Kramer et al., 1997). Fatty acids were transesterified to methyl esters based on methods reported by Kramer et al. (1997) that included incubation first in sodium methoxide (0.5 M in methanol) for 10 min at 50°C followed by incubation in 5% methanolic HCl for 10 min at 70°C .

Apparent digestibility of DM, CP, ADF, NDF, and fat were calculated by the marker ratio technique (Schneider and Flatt, 1975).

In Situ Rate and Extent of Digestion. Rate, extent, and lag of DM and NDF digestibility of corn silage were measured by the Dacron bag technique (Nocek, 1988). A single sample of corn silage was collected at the beginning of the trial, dried at 55°C for 48 h, and ground to pass a 2-mm screen (Wiley mill, A. H. Thomas). Approximately 5.5 g (as-is) was weighed into preweighed polyester bags (10×20 cm) with an average pore size of $53 \pm 10 \mu\text{m}$ (Bar Diamond, Inc., Parma, ID). Bags were incubated in the rumen via cannula at intervals of 0, 4, 8, 12, 18, 24, 36, 48, and 72 h starting on d 25 of each period. All bags were removed simultaneously. After removal, bags were placed in ice water, washed under running tap water by hand, and washed without soap in a washing machine on delicate/cold cycle to remove ruminal fluid. Upon removal, bags were oven-dried for 48 h at 55°C , then weighed to determine DM residue. The undigested residue was analyzed for NDF (Van Soest et al., 1991). The equation for the determination of lag time and rate of DM and NDF

digestion was the same as used in the study by Mertens and Ely (1982):

$$R = D_o e^{-K(t-L)} + U \text{ when } t > L \text{ and } R = D_o + U \text{ when } 0 < t < L,$$

where R = DM or NDF residue at time t after incubation, D_o = slowly digestible fraction at $t \leq L$ and $D_o = R - U$, K = digestion rate constant, L = discrete lag time, and U = indigestible fraction at 72 h of in situ fermentation.

Digestion rate constants and discrete lag times were calculated with the nonlinear models procedure of SAS (Release 8.2; SAS Institute Inc., Cary, NC) using the method of Marquardt (1963).

Sampling and Analysis of Ruminal Fluid. Ruminal fluid (~ 150 mL) was collected hourly for 8 h starting at feeding on d 15 of each period using the ruminally fistulated cows. Using a suction strainer system consisting of a 1-L, side-armed flask equipped with a rubber bulb and a perforated hard plastic tube inserted into the ruminal fluid, samples were collected from at least 6 locations within the rumen. The pH was measured immediately upon collection (pH meter, model 15; Fisher Scientific). A subsample of about 30 mL was acidified with 50% sulfuric acid to a pH between 2 and 3 and centrifuged at $5400 \times g$ for 20 min. The supernatant was collected and frozen immediately at -20°C until further analysis for VFA on a 4% Carbo-max 80/120 BDA (Supelco Inc., Bellefonte, PA) column in a gas chromatograph (Autosystem XL, Perkin Elmer Inc., Norwalk CT). Prior to injection unto column, samples were centrifuged at $5,000 \times g$ for 30 min and filtered with a high-affinity protein syringe-driven filter unit (Millex SLAA025LS, Fisher Scientific). The gas chromatograph was set to a flow rate of 30 mL/min of N, an injection port temperature of 200°C , oven at 175°C , and the flame-ionizing detector at 450°C .

Protozoa numbers were determined as described by Dehority (1984). Ruminal fluid (10 mL) was collected every 2 h for 8 h starting at feeding, mixed with 10 mL of 50% formalin, and stored at room temperature. Brilliant green dye (2 drops) was added to 1-mL aliquots of preserved ruminal fluid and allowed to stand overnight. After staining, 9 mL of 30% glycerol were added and 1 mL of the diluted sample was pipetted into a Sedgewick-Rafter counting chamber (1-cm^3 volume). Protozoa were counted at a magnification of $100\times$. The goal was to have approximately 100 to 150 protozoa per 50 grids. Further dilutions were made with 30% glycerol if needed.

Statistical Analysis. Measurements of feed intake, milk production and composition, in situ lag, rate, and

extent of digestion, apparent digestibility, pH of urine and feces, rectal temperatures, plasma glucose, plasma urea nitrogen, and microbial protein synthesis were analyzed by the GLM procedure of SAS (Release 8.2; SAS Institute Inc.). The model was

$$Y_{ijkl} = \mu + \alpha_i + \beta_{ij} + \gamma_k + \lambda_l + (\alpha\lambda)_{il} + (\alpha\gamma)_{ik} + \varepsilon_{ijkl}.$$

Y_{ijkl} = response variable in square i in period k in treatment l for cow j , μ = overall mean, α_i = effect of square i , β_{ij} = effect of cow j within square i , γ = effect of period k , λ = effect of treatment l , $(\alpha\lambda)_{il}$ = effect of interaction of square i with treatment l , $(\alpha\gamma)_{ik}$ = effect of interaction of square i with period k , and ε_{ijkl} = residual effect of i , j , k , and l . The error term for square was cow within square.

Results are reported as least squares means. Significance was determined at $P < 0.05$ and a tendency toward significance at $P < 0.10$. The fatty acid profile of milk samples were analyzed as an incomplete Latin square design because of missing values. The model was the same as above except that square, square by treatment interaction, and square by period interaction were deleted. Repeated measures of hour for ruminal pH, protozoa numbers, and VFA data were analyzed by the Mixed (Littell et al., 1996) procedure of SAS (Release 8.2; SAS Institute Inc.). The model was similar to that described previously except that hour and interaction of hour and treatment were included. Covariance structures were tested to determine the best fit for each dependent variable. Single degree of freedom contrasts for linear and quadratic effects of treatment were tested. A reduced model was used, pooling the square by period interaction with the error term if the square by period interaction was $P > 0.25$. Then, if the treatment by square interaction was $P > 0.25$, it was pooled with the error term as well (Bancroft, 1968).

Experiment 2

Lactating dairy cows ($n = 190$, 210 ± 87 DIM) at the University of Florida's research farm were divided randomly into 2 groups and assigned a diet of either 0 or 1.8% CFO (DM basis) in a 2-period reversal trial. Each period was 28 d long, of which the first 14 d served as an adjustment period and the second 14 d was used for data collection. The CFO was mixed with liquid molasses and fed as part of a TMR as described in experiment 1. Molasses and citrus pulp were adjusted to maintain similar dietary concentrations of sugar (Table 3). Collection of feed samples and feed analyses were as described in experiment 1. Housing and milking management were as described for cows in experiment 1. Cows were fed 3 times daily in ad libitum amounts.

Average daily milk production during the 10 d before initiation of dietary treatments and the fat and protein concentrations of the milk measured on milk samples collected during this time were used as covariates in the statistical analyses. Cows were fed a common diet during the covariate period. Milk samples were collected on d 26 of each period and analyzed for fat and protein as described in experiment 1. The following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk}.$$

Y_{ijk} = response variable for cow i in period j fed treatment k , μ = overall mean, α_i = effect of cow i , β_j = effect of period j , and γ = effect of treatment k , and ε_{ijk} = residual effect of i , j , and k . The GLM procedure of SAS (Release 8.2; SAS Institute Inc.) was used. Results are reported as least squares means. Significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Experiment 1

Intake Response and Apparent Digestibility.

Dry matter intake (in kg/d and as a % of BW) increased (linear component, $P = 0.01$ and 0.03 , respectively) with increasing dietary concentration of CFO (Table 4). DePeters et al. (1987) also reported an increase in DMI when cows were fed a diet of 3.5% yellow grease compared with those not fed yellow grease. Others have reported increased DMI as fats containing less unsaturated fats were fed. As partially hydrogenated tallow replaced tallow, DMI increased from 23.1 to 24.7 kg/d (Pantoja et al., 1996). In agreement was the work of Pantoja et al. (1994), in which DMI increased as partially hydrogenated tallow replaced tallow, which in turn replaced a tallow-canola oil mixture (20.8, 18.8, and 17.8 kg/d). The fatty acid profile of CFO is composed of less polyunsaturated fatty acids than whole cottonseeds (20 vs. 52%) and may account for the change in DMI caused by intake of CFO. When marine fish oils were a source of supplemental fat, DMI was depressed when the fish oil constituted $\geq 1.6\%$ of dietary DM (AbuGhazaleh et al., 2002; Lacasse et al., 2002; Whitlock et al., 2002) but was unchanged when fed at $\leq 1\%$ of dietary DM (Donovan et al., 2000; Keady et al., 2000). Most studies have reported no effect on DMI by feeding yellow grease (Martinez et al., 1991; Nianogo et al., 1991; Avila et al., 2000) or tallow (Eastridge and Firkins, 1991; Grummer et al., 1993; Smith et al., 1993) but others have reported a depression in DMI when tallow was fed at 2% of dietary DM (Onetti et al., 2001, 2002; Ruppert et al., 2003). The mechanisms

Table 3. Ingredient and chemical composition of experimental diets containing catfish oil (CFO) fed to lactating Holstein cows in summer in experiment 2

Item	0% CFO	1.8% CFO
Ingredient		
Corn silage, % of DM	23.1	23.1
Sorghum silage, % of DM	8.9	8.9
Alfalfa hay, % of DM	10.9	11.0
Cottonseed hulls, % of DM	5.4	5.4
Corn meal, % of DM	20.8	20.8
Soybean meal, % of DM	8.8	8.9
Citrus pulp, % of DM	5.6	4.2
Extruded soybean meal, ¹ % of DM	7.1	7.1
Liquid molasses, ² % of DM	5.2	0.0
Liquid molasses + CFO, ³ % of DM	0.0	6.5
Mineral and vitamin mix, ⁴ % of DM	3.7	3.7
Sodium bicarbonate, ⁵ % of DM	0.5	0.5
Chemical		
CP, % of DM	16.3	16.3
NDF, % of DM	29.8	29.5
ADF, % of DM	18.5	18.7
Fat, % of DM	3.2	4.6
Starch, % of DM	23.5	23.5
Sugars, % of DM	9.3	9.7
NE _L , Mcal/kg	1.60	1.67
Ca, % of DM	1.32	1.34
P, % of DM	0.39	0.39
Mg, % of DM	0.36	0.38
K, % of DM	1.46	1.41
Na, % of DM	0.87	0.95
S, % of DM	0.22	0.24
Cu, mg/kg of DM	26	37
Fe, mg/kg of DM	211	219
Mn, mg/kg of DM	44	86
Zn, mg/kg of DM	114	128

¹SoyPlus, West Central Soy, Ralston, IA.²United States Sugar Corp., Clewiston, FL.³CFO supplied by Protein Products, Inc., Gainesville, GA, to United States Sugar Corp. for mixing at 27.4% (DM basis).⁴Mineral and vitamin mix contained 26.4% CP, 1.74% fat, 10.15% Ca, 0.90% P, 3.1% Mg, 8.6% Na, 5.1% K, 1.5% S, 4.1% Cl, 2,231 mg of Mn/kg, 1,698 mg of Zn/kg, 339 mg of Fe/kg, 512 mg of Cu/kg, 31 mg of Co/kg, 26 mg of I/kg, 7.9 mg of Se/kg, 67,021 IU of vitamin A/kg, 19,845 IU of vitamin D/kg, and 357 IU of vitamin E/kg (DM basis).⁵Church and Dwight Inc., Piscataway, NJ.

by which supplemental fat sometimes depresses feed intake are not clear, but could involve effects of fat on ruminal fermentation and gut motility, acceptability of diets containing added fat, release of gut hormones, and oxidation of fat in the liver (Allen, 2000).

Accompanying the increase in DMI, apparent digestibility of DM increased (linear component, $P = 0.001$) as intake of CFO increased (Table 4). Similar to this study, apparent digestibility of DM or OM increased when cows were fed yellow grease (Jenkins and Jenny, 1989; Nianogo et al., 1991), tallow or tallow-canola oil blend (Pantoja et al., 1994), or marine fish oil (Doreau and Chilliard, 1997; Keady et al., 2000). Part of this increase in DM digestibility in the present study was caused by an increased digestibility of the fat fraction of the diet; that is, the fat in CFO was more digestible than that in whole cottonseeds and the other ingredients. Others have reported a greater apparent ether extract digestibility when cows were fed marine fish oil (Doreau and Chilliard, 1997), yellow grease (DePeters et al., 1987), or tallow (Smith et al., 1993; Ruppert et al., 2003).

The apparent digestibility of CP also increased (linear component, $P = 0.01$) as intake of CFO increased (Table 4). Soybean meal increased in the diet along with increasing concentration of CFO; therefore, this increase in extent of CP digestion may have occurred simply because the protein in soybean meal was more digestible than that in whole cottonseed (NRC, 2001). However others have reported an improvement in apparent CP digestibility when lactating dairy cows were fed yellow grease (Nianogo et al., 1991) or tallow (Lewis et al., 1999) and the fat sources replaced grains.

Apparent digestibilities of NDF and ADF increased (linear component, $P = 0.02$) as intake of CFO increased (Table 4). Likewise, Doreau and Chilliard (1997) reported an increase in NDF digestibility when marine fish oil was supplemented at 370 g/d. These

Table 4. Dry matter intake, apparent digestibility coefficients of DM, CP, NDF, ADF, and fat, BW change, and rectal temperatures (RT) of lactating Holstein cows ($n = 12$ per treatment) fed catfish oil (CFO) in summer in experiment 1

Measure	Dietary treatment				<i>P</i> -value	
	0% CFO	1.5% CFO	3.0% CFO	SEM	Linear	Quadratic
DMI, kg/d	23.0	24.4	25.4	0.6	0.01	0.81
DMI, % of BW	3.2	3.4	3.5	0.08	0.03	0.94
Apparent digestibility, %						
DM	69.9	74.5	75.3	0.8	0.001	0.08
CP	72.2	75.8	76.2	1.0	0.01	0.19
NDF	51.9	59.0	58.5	1.7	0.02	0.10
ADF	41.5	50.4	49.0	2.0	0.02	0.06
Fat	75.6	84.2	87.8	1.3	0.001	0.15
BW change, kg/27 d	25.3	35.0	31.0	6.3	0.55	0.42
RT at 0430 h, °C	38.2	38.2	38.4	0.04	0.03	0.13
RT at 1630 h, °C	38.7	38.8	38.7	0.04	0.99	0.08

Table 5. Milk production and composition of lactating Holstein cows (n = 12 per treatment) fed catfish oil (CFO) in summer in experiment 1¹

Measure	Dietary treatment			SEM	P-value	
	0% CFO	1.5% CFO	3.0% CFO		Linear	Quadratic
Milk, kg/d	29.0	29.0	29.5	0.5	0.52	0.72
Milk fat, %	3.57	3.60	3.48	0.14	0.70	0.71
Milk fat, kg/d	1.03	1.02	1.01	0.05	0.81	0.99
Milk protein, %	3.21	3.18	3.23	0.02	0.48	0.27
Milk protein, kg/d	0.91	0.91	0.94	0.02	0.39	0.54
4% FCM, kg/d	27.0	27.0	27.0	0.8	0.96	0.94
SCC × 1,000/mL	154	95	109	0.5	0.51	0.49

¹Average DIM and SD were 195 ± 27 at the beginning of the study.

findings are in contrast to other reports of supplemental fat depressing fiber digestibility (Nianogo et al., 1991; Smith et al., 1993). It has often been suggested that the negative effect of dietary lipids on intake is mainly caused by a depressive effect on ruminal digestion or to a low palatability of fat supplements. It was not the case in this study, in which CFO enhanced both DMI and digestibility. The extent that fat may interfere with digestion depends on the amount of fat fed and the source. Increasing esterification or saturation of fats generally lessens its negative effects on ruminal fermentation (Palmquist and Jenkins, 1980).

These cows in the latter stages of their lactation were gaining ≥0.9 kg/d (Table 4). No difference was detected in BW gain among treatment groups; however, cows fed CFO had numerically greater BW gain than cows fed the control diet. When the 3 rations and respective DMI and milk yields and composition were entered into the NRC ration evaluation software (NRC, 2001), the calculated daily gains were 0.9, 1.5, and 2.0 kg/d. The daily gains from the current trial were 0.94, 1.3, and 1.15 kg/d for cows fed the 0, 1.5, and 3.0% CFO diets, respectively. Gains for cows fed diets containing 0% and 1.5% CFO match well with the calculated gains. DePeters et al. (1987) reported greater BW gain by cows in late compared with early lactation when fed yellow grease. Increased DMI and apparent digestibility when consuming CFO could be expected to result in greater gain, milk production, or both. It is unclear why these responses did not occur.

Morning rectal temperatures increased (linear component, $P = 0.03$) as cows were fed increasing amounts of CFO, whereas afternoon rectal temperatures tended ($P = 0.08$) to increase quadratically (Table 4). These increases were most likely caused by increased DMI of cows fed CFO.

Milk Production and Composition. Milk production (Table 5) was unchanged by CFO in the diet. This is somewhat surprising because cows fed CFO consumed increasing amounts of digestible DM (Table 4). Donovan et al. (2000) reported that milk yield increased for cows

fed 1% marine fish oil diets, but milk yield decreased when fish oil was fed at 3% of dietary DM. Keady et al. (2000) also reported an increase in milk yield when marine fish oil was fed at 0.9, 1.9, and 3.1% of dietary DM using diets high in grass silage. However, others reported a reduction in milk production when feeding diets of 3.7% fish oil (Lacasse et al., 2002) or 2% fish oil (Whitlock et al., 2002). Concentration and yield of milk fat and protein as well as somatic cell counts were unchanged by CFO (Table 5).

Fatty Acid Composition of Milk Fat. Short- and medium-chain fatty acids, C4:0 to C12:0, in milk fat were not affected by inclusion of CFO in the diet (Table 6). We expected that the supply of long-chain fatty acids to the mammary gland was not changed appreciably by replacing whole cottonseed with CFO. Therefore, it was not unexpected that concentrations of C4 to C12 fatty acids were similar across diets. Concentration of C14:0 tended to increase (linear component, $P = 0.08$) in milk fat of cows fed increasing amounts of CFO. The concentrations of fatty acids C14:1 and C16:1 increased (linear component, $P = 0.004$) in milk fat of cows fed increasing amounts of CFO. Concentration of C18:0 in milk fat decreased (linear component, $P = 0.02$) and the concentration of C18:2 tended to decrease (linear component, $P = 0.07$) with increasing inclusion of CFO in the diet. An increase (linear component, $P = 0.11$) in the concentration of C20:5 caused by CFO supplementation came close to a statistical tendency. These effects were likely caused by the differences in fatty acid profiles between CFO and whole cottonseed. Whole cottonseed oil contains greater concentrations of 18-carbon fatty acids. Concentrations of *cis*-9, *trans*-11 C18:2 and *trans*-10, *cis*-12 C18:2 increased numerically with increasing intake of CFO but large standard errors prevented these responses from being significant. Others (AbuGhazaleh et al., 2002; Whitlock et al., 2002) reported that the feeding of marine fish oil increased the conjugated linoleic acid concentration of milk fat. A lack of response in the current study was likely caused by a lower concentration of eicosapentaenoic acid and

Table 6. Fatty acid composition (g/100 g of identified fatty acids) of milk fat of lactating Holstein cows (n = 8 to 9 per treatment) fed catfish oil (CFO) in summer in experiment 1

Fatty acid	Dietary treatment			SEM	P-value	
	0% CFO	1.5% CFO	3.0% CFO		Linear	Quadratic
C4:0	3.51	3.52	3.36	0.11	0.32	0.59
C6:0	2.37	2.49	2.41	0.06	0.62	0.27
C8:0	1.28	1.38	1.36	0.05	0.28	0.45
C10:0	2.88	3.13	3.10	0.15	0.31	0.51
C11:0	0.03	0.05	0.04	0.01	0.37	0.47
C12:0	3.22	3.55	3.61	0.19	0.16	0.61
C14:0	10.32	11.21	11.38	0.39	0.08	0.51
C14:1	1.28	1.51	1.63	0.07	0.004	0.52
C15:0	0.82	0.94	0.93	0.05	0.13	0.31
C16:0	29.05	28.93	30.04	0.56	0.22	0.43
C16:1	1.43	1.59	1.84	0.08	0.004	0.68
C17:0	0.48	0.47	0.46	0.02	0.23	0.89
C18:0	10.31	9.10	7.89	0.62	0.02	0.99
C18:1	20.93	19.27	19.46	0.80	0.21	0.41
<i>trans</i> -C18:1	1.57	2.15	1.83	0.27	0.48	0.25
C18:2	3.32	3.05	3.07	0.09	0.07	0.27
C18:3	0.33	0.32	0.35	0.02	0.44	0.50
<i>cis</i> -9, <i>trans</i> -11 CLA	0.008	0.009	0.011	0.005	0.62	0.98
<i>trans</i> -9, <i>cis</i> -11 CLA	0.05	0.05	0.05	0.004	0.71	0.81
<i>trans</i> -10, <i>cis</i> -12 CLA	0.004	0.009	0.010	0.005	0.40	0.73
C20:0	0.18	0.19	0.18	0.01	0.94	0.75
C21:0	0.06	0.06	0.07	0.003	0.10	0.46
C22:0	0.19	0.19	0.18	0.01	0.20	0.51
C22:1	0.20	0.19	0.20	0.01	0.96	0.31
C20:5	0.02	0.02	0.03	0.005	0.11	0.38
C24:0	0.02	0.03	0.02	0.007	0.66	0.39
C22:6	0.02	0.04	0.03	0.03	0.84	0.70

docosahexaenoic acid in CFO compared with that in marine fish oil (Whitlock et al., 2002).

Ruminal Fermentation. Diet by hour interactions were not observed for any of the ruminal measurements. Ruminal fluid pH decreased (linear component, $P = 0.001$) when cows were fed diets of increasing concentration of CFO (Table 7). This lower pH may have resulted from greater intake and digestibility of DM of diets with increasing concentration of CFO (Table 4). Grummer et al. (1993) observed a linear reduction in

ruminal pH with increasing concentrations of tallow in the diet (0, 1, 2, and 3%). Authors indicated that this reduction reflected stimulation rather than inhibition of fermentation.

Total VFA concentrations in ruminal fluid were similar across diets (Table 7). This is somewhat surprising because ruminal fluid pH decreased linearly as intake of CFO increased. The VFA are absorbed from the rumen at a faster rate with decreasing pH. Therefore, increased production of VFA accompanied by increased

Table 7. Least squares means for VFA concentration, pH, microbial protein production, and protozoa numbers in ruminal fluid of lactating Holstein cows (n = 6 per treatment) fed catfish oil (CFO) in summer in experiment 1¹

Measure	Dietary treatment			SEM	P-value	
	0% CFO	1.5% CFO	3.0% CFO		Linear	Quadratic
Ruminal fluid pH	6.41	6.20	6.15	0.07	0.001	0.12
Acetate, molar %	64.5	64.2	63.4	0.77	0.02	0.58
Propionate, molar %	19.4	20.0	20.4	0.53	0.02	0.78
Butyrate, molar %	12.2	12.4	12.5	0.43	0.08	0.37
Isobutyrate, molar %	0.77	0.62	0.68	0.04	0.01	0.001
2-Methylbutyrate, molar %	1.30	1.08	1.17	0.08	0.04	0.003
Valerate, molar %	1.32	1.26	1.37	0.09	0.43	0.07
Isovalerate, molar %	0.51	0.38	0.44	0.04	0.01	0.001
Total VFA, mM	109.1	112.6	111.9	12.0	0.69	0.73
Acetate:propionate ratio	3.38	3.22	3.18	0.13	0.01	0.35
Microbial protein, g/d	298	342	285	11	0.42	0.02
Protozoa, $\times 10^3$ /mL	7.6	7.1	7.7	0.6	0.86	0.23

¹Nine hourly samples of ruminal fluid were collected per cow in each of 3 periods for VFA and pH measures. Treatment by time interactions were not significant.

Table 8. In situ lag, rate, and extent of DM and NDF digestion of corn silage by lactating Holstein cows (n = 6 per treatment) fed catfish oil (CFO) in summer in experiment 1

Measure	Dietary treatment			SEM	P-value	
	0% CFO	1.5% CFO	3.0% CFO		Linear	Quadratic
DM						
Lag, h	2.1	1.8	4.9	0.8	0.08	0.17
Rate, h ⁻¹	0.022	0.022	0.029	0.002	0.12	0.40
72 h extent, %	83.7	81.7	83.2	1.4	0.85	0.43
NDF						
Lag, h	9.7	8.8	10.8	1.5	0.82	0.73
Rate, h ⁻¹	0.023	0.024	0.029	0.001	0.04	0.24
72 h extent, %	65.5	60.4	64.3	3.2	0.83	0.38

absorption of VFA in the rumen of cows fed CFO may have resulted in no net change in VFA concentration among cows fed the 3 diets. The molar proportion of acetate in ruminal fluid decreased (linear component, $P = 0.02$) with increasing concentrations of CFO in the diet, whereas the molar proportion of propionate increased (linear component, $P = 0.02$) by adding CFO in the diet. As a result, the acetate to propionate ratio in ruminal fluid decreased (linear component, $P = 0.01$) as more CFO was fed to cows. Doreau and Chilliard (1997) reported a decrease in molar proportion of acetate and an increase in propionate in ruminal fluid of cows fed diets supplemented with 370 g of menhaden fish oil/d. Similar results were obtained by Onetti et al. (2001) and Lewis et al. (1999) when tallow was the source of fat. Molar proportions of butyrate tended to increase (linear component, $P = 0.08$) with CFO. Molar proportions of isobutyrate, 2-methylbutyrate, valerate, and isovalerate decreased or tended to decrease in ruminal fluid of cows fed 1.5% CFO, but no further decrease was detected when cows were fed diets of 3% CFO (quadratic component, $P < 0.10$; Table 7). It is unlikely that these small shifts in VFA proportions will have a meaningful physiological effect.

Onetti et al. (2001) reported a decrease in protozoa number/mL of ruminal fluid as tallow or choice white grease increased in the diet from 0 to 4% of dietary DM. Their study reported no difference in protozoa numbers between sources of fat. In the current study, protozoa numbers in ruminal fluid were not changed by the feeding of CFO (Table 7).

Microbial protein yield (g/d) increased in cows fed diets of 1.5% CFO but returned to that of controls

when cows were fed diets of 3.0% CFO (quadratic component, $P = 0.02$; Table 7). A small increase in dietary fat may improve bacterial growth if bacteria can incorporate more dietary fatty acids directly and reduce the need to synthesize them. However, feeding more fat may have slightly negative effects on bacterial metabolism and the advantage is lost. Flow of bacterial N from the duodenum of lactating dairy cows tended to increase as saturation of supplemental fat decreased (Pantoja et al., 1994). Bacterial growth may have benefited because the iodine value of CFO is much less than that of whole cottonseed oil, although this did not carry over to cows fed the 3% CFO diet.

In Situ DM and NDF Digestion. In situ lag, rate, and extent of digestion of corn silage DM was unchanged by feeding CFO (Table 8). In situ lag time of NDF digestion was unchanged by CFO, but the rate of NDF digestion increased (linear component, $P = 0.04$) with increasing amounts of CFO in the diet. However, the extent of NDF digestion at 72 h was similar across diets (average 63.4%). Increased rate of NDF digestion likely contributed to the increase in DMI and apparent total tract digestion of NDF observed in cows fed CFO (Table 4).

Unsaturated fatty acids can cause an alteration in the ruminal ecosystem because of the suppression of methanogenic (and to a lesser extent cellulolytic) bacteria and protozoa (Van Soest, 1994). A decrease in methane production results in an alteration of the ruminal fermentation, leading to an increase in propionate production to maintain the ruminal fermentation balance. The possible depression of methane producers in the rumen and no change in protozoa numbers by

Table 9. Concentrations of plasma urea, glucose, and insulin of lactating Holstein cows (n = 12 per treatment) fed catfish oil (CFO) in summer in experiment 1

Measure	Dietary treatment			SEM	P-value	
	0% CFO	1.5% CFO	3.0% CFO		Linear	Quadratic
Urea, mg/100 mL	11.6	11.0	12.0	0.4	0.61	0.20
Glucose, mg/100 mL	57.8	55.1	56.0	1.1	0.30	0.24
Insulin, ng/mL	0.55	0.53	0.57	0.04	0.74	0.56

Table 10. Milk production and composition of lactating Holstein cows ($n = 190$ per treatment) fed catfish oil (CFO) in summer in experiment 2

Measure	Dietary treatment		SEM	<i>P</i> -value	
	0% CFO	1.8% CFO		Diet	Period
DMI, kg/d	19.6	19.6			
Milk, kg/d	30.8	32.0	0.2	0.001	0.001
Milk fat, %	3.29	3.37	0.05	0.23	0.65
Milk fat, kg/d	1.02	1.09	0.02	0.01	0.01
Milk protein, %	2.89	2.94	0.015	0.01	0.0001
Milk protein, kg/d	0.88	0.94	0.008	0.001	0.001
4% FCM, kg/d	27.7	29.3	0.3	0.001	0.001

CFO inclusion in this study may have created a space in the microbial mass that cellulolytic bacteria occupied, thus increasing fiber digestion.

Blood Metabolites. Concentrations of plasma urea, glucose, or insulin were not affected by CFO supplementation (Table 9). Least squares means and SEM were 11.5 and 0.4 mg/100 mL, 56.3 and 1.1 mg/100 mL, and 0.55 and 0.04 ng/mL for urea, glucose, and insulin, respectively. Others have reported no change in plasma glucose concentration when fat was added to the diet (Ruppert et al., 2003). Palmquist and Jenkins (1980) noted in their review that high-fat diets can result in an inability of insulin to stimulate glucose utilization by tissues, thus causing an increase in plasma glucose concentration. As found in the current study, Smith et al. (1993) and Ruppert et al. (2003) reported no effect of fat supplementation on BUN concentration.

Experiment 2

Cows fed CFO produced 1.2 kg more milk/d ($P = 0.001$; Table 10). Using the NRC (2001) ration evaluator software, NE_L allowable milk was predicted as 30.8 and 32.2 kg/d for cows fed the 0 and 1.8% CFO diets, respectively, with the increase caused by increased energy concentration of the diet. This is a very close match with the values reported here. An increase in milk production caused by the feeding of CFO may have been detected in this study compared with the first study because of the greater number of cows used in experiment 2 (380 vs. 36 cow observations), which provided greater statistical sensitivity. In addition, the fat concentrations of the diets were increased from 3.2 to 4.6% (Table 3) by the addition of CFO to the molasses, which allowed greater energy intake for the production of milk. Dry matter intake did not differ between the 2 groups at 19.6 kg/d. Mean concentration of milk fat was 3.33% and did not differ between treatment groups. Feeding CFO resulted in greater concentrations of protein in milk (2.94 vs. 2.89%) that may have been caused by greater production of microbial protein in the

rumen as found in experiment 1 (Table 7). Because of greater production of milk, production of fat, protein, and FCM were greater for cows fed CFO.

CONCLUSIONS

Catfish oil mixed with liquid molasses and fed to late-lactation Holstein cows ($n = 12$ in a Latin square design) at 1.5 and 3% of dietary DM stimulated DMI and digestibility. Fermentation in the rumen was not affected negatively by feeding CFO based upon improved in situ digestion rate of NDF, only moderate changes in VFA, and improved synthesis of microbial protein. Although the production and composition of milk was unchanged by feeding CFO in this study, the increase in feed intake and digestibility hold promise that milk production could be increased in future studies. In a second study involving 190 Holstein cows, dietary CFO increased milk production by 1.2 kg/d when fed at 1.8% compared with 0% of dietary DM. This increase may have been caused by greater energy density of the CFO diet. Catfish oil appears to be an acceptable oil source for lactating cow diets (up to 3% of dietary DM).

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