In Vitro Degradation of Choline from Selected Feedstuffs and Choline Supplements[1,2]

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ABSTRACT

The objective of this experiment was to determine the extent of in vitro degradation of choline from barley, corn, corn gluten meal, cottonseed meal, fish meal, soybean meal, alfalfa hay, timothy hay, choline chloride, and choline stearate. During four individual fermentation runs, samples were incubated in vitro for .25, 1, 3, 6, 12, 18, 24, 36, and 48 h with an inoculum mixture containing rumen fluid obtained from a rumen-fistulated dairy cow fed 17.5% corn silage and 28.7% grass silage and 53.8% concentrate diet. Because of their low choline content (less than .68 mg/g) corn, corn gluten meal, alfalfa hay, and timothy hay gave erratic values for choline disappearance for different fermentation runs and times of incubation although disappearance tended to increase with time. Data for the rest of the feeds and choline supplements were analyzed using nonlinear regression procedure to obtain estimates of potentially degradable choline, rapidly degradable choline, and the rate of choline degradation in vitro. The mean estimates of rumen degradable choline (%) were 79.4, 84.7, 82.9, 83.8, 98.0, and 98.6 for barley, cottonseed meal, fish meal, soybean meal, choline stearate, and choline chloride, respectively. The results suggest that incorporating choline-rich feedstuffs in diets can only marginally increase the postruminal flow of choline in dairy cows.

INTRODUCTION

Phosphatidylcholine is the major form of naturally occurring choline in feedstuffs (13), although a small amount of free choline can also exist in plant materials (5, 17). Rumen microorganisms degrade dietary phosphatidylcholine to choline and phosphodiglycerides (13). They convert the released choline and any available free choline into methane via an intermediate compound trimethylamine (12, 13). Some of the free choline in the rumen is utilized by rumen protozoa for the synthesis of protozoal phosphatidylcholine (3). However, the majority of protozoal populations (65%) die and are digested in the rumen (9). Thus, a very limited amount of dietary choline is available postruminally in the ruminant.

Synthetic choline is also extensively degraded in the rumen. Atkins et al. (1) reported that 87.5% of ruminally administered dose (26 g) of choline was degraded in the rumen. The degradation mechanism of choline in the rumen could not be overwhelmed by increasing the choline supplementation up to 20 g/kg ration DM (14).

It has been generalized that essentially all dietary choline is degraded in the rumen (8). Because feedstuffs differ in their DM digestibility, chemical composition, and choline content, it was hypothesized that the extent of rumen degradation of choline from various feedstuffs is not similar in the rumen. If some choline-rich feedstuffs also contained less rumen degradable choline, feeding such feeds would increase the postruminal availability of choline. The objective of this experiment was to determine the extent of in vitro degradation of choline from selected feedstuffs and synthetic choline supplements.

MATERIALS AND METHODS

Barley, corn, corn gluten meal, cottonseed meal, soybean meal, fish meal, alfalfa hay,

Received August 1, 1988.
Accepted April 20, 1989.
[1]Scientific Article Number A-4778.
[2]Contribution Number 7784 of the Maryland Agricultural Experiment Station.

timothy hay, oven-dried corn silage (92% DM), choline chloride, and choline stearate (Syntex Agribusiness, Inc., SSS, Springfield, MO; added to corn cob meal) were tested. Feed samples were ground using a 2-mm screen. Samples were digested by simmering in 50 ml of 15% nitric acid for 3 h (2). Choline content of the digest was determined by the colorimetric enzymatic procedure of Takayama et al. (18) except that phospholipase-D was omitted.

In vitro choline disappearance was determined by a method similar to the DM digestibility procedure of Tilly and Terry (19) using a constant temperature, shaking water bath at 39°C. For each of the four fermentation runs, samples of feedstuff (1 g) and choline supplements (.05 to .1 g) were placed in 100-ml Nalgene tubes, fitted with one-way gas valves, containing known amounts (.5 to 1 g) of corn silage DM. A set of control tubes contained no other samples but corn silage. Prior to the addition of inoculum mixture, 25 ml of McDougall's buffer solution (10) were added to each tube, and tubes were placed in the water bath at 39°C during preparation of inoculum mixture. Rumen fluid for inoculum mixture was obtained from a rumen-fistulated dairy cow fed a diet containing 17.5% corn silage, 28.7% grass silage, and 53.8% concentrate. Rumen fluid was collected in a prewarmed, insulated container by squeezing rumen contents through four layers of cheesecloth. It was transported to the laboratory, strained through four more layers of cheesecloth, and mixed with McDougall's buffer solution (10) at a ratio of 1:1.5. Inoculum mixture was fluxed continuously with carbon dioxide to maintain the anaerobic conditions until added (25 ml) to the incubation tubes. Sample tubes (1 tube/sample/incubation time) were incubated under anaerobic conditions for .25, 1, 3, 6, 12, 18, 24, 36, and 48 h.

Upon completion of each fermentation run, 1 ml of saturated mercuric chloride solution was added to sample tubes. Liquid from the tubes was aspirated off and the volume of liquid was recorded. A 10-ml sample of aspirated liquid was frozen for choline analysis. Solid residues after incubation were oven dried to complete dryness (65°C for 72 h) and weighed. Solid residues and liquid samples were analyzed for their choline content using the method mentioned earlier.

The final calculations of the data are based on the assumption that the rate and extent of choline disappearance from test feeds were not affected by incubation with corn silage. Corn silage being low in choline content (.38 mg/g) would have little effect on choline degradation of most feeds used except for those containing less than 1.0 mg/g choline. Data from individual fermentation runs were analyzed using nonlinear regression procedure based on Marquardt's compromise (4) of the Statistical Analysis System (SAS). The following model described by Erdman and Vandersall (7) was used:

$$D = R + (P - R) \times (1-e^{-kt}) + \text{random error}$$

where:

- D = Percentage of choline degraded at incubation time = t;
- R = Percentage of choline instantaneously degraded at t = 0 h;
- P = Percentage of total potentially degradable choline, including rapidly degradable choline that will be degraded when given sufficient time for digestion; and
- k = a rate constant for disappearance of fraction, (P − R).

Parameter estimates were also used to estimate the amount of choline that would be degraded in the rumen (Q), using the equation:

$$Q = R + (P - R) \times k / (k + .05)$$

where added constant .05 is the assumed rate of outflow from the rumen (7). The estimates of P, R, k, and Q were further analyzed using General Linear Models procedure of SAS. The model included sample and fermentation run effects. Where sample effects were significant ($P<.05$), mean estimates were further separated using Student-Newman-Keuls' procedure (16).

RESULTS AND DISCUSSION

Choline content of all samples used is presented in Table 1. Among feed samples, fish meal contained the highest choline content of 4.17 mg/g. Soybean meal and cottonseed meal contained 2.95 and 2.60 mg choline/g, respectively. Corn contained much less choline than barley did (.68 vs. 1.84 mg/g). Corn gluten

TABLE 1. Choline content of samples used in in vitro experiment.

Sample	Choline, mg/g	
	X	SD
Corn silage	.38	.09
Barley	1.84	.05
Corn	.68	.10
Corn gluten meal	.60	.11
Cottonseed meal	2.60	.31
Fish meal	4.17	.57
Soybean meal	2.95	.23
Alfalfa hay	.43	.44
Timothy hay	.36	.08
Choline stearate[1]	162.8	17.57
Choline chloride[1]	357.9	32.61

[1]Syntex Agribusiness, Inc., SSS, Springfield, MO 65805.

meal was lower in choline than was corn, as expected. Choline values obtained for corn, corn gluten meal, cottonseed meal, and fish meal from this experiment are comparable to those listed by National Research Council (11), except choline for barley was higher. Alfalfa hay, timothy hay, and corn silage were very low in choline. Choline chloride and choline stearate contained 35.8 and 16.3% choline, which was nearly identical to values specified by the manufacturer.

The mean percentages of choline disappearance in vitro from all samples at various times of incubation are presented in Table 2. Mean choline disappearances for corn, corn gluten meal, alfalfa hay, and timothy hay did not increase with time of incubation. Also, observed choline disappearances for these samples were highly variable between different fermentation runs and for various times of incubations, which is reflected in higher standard errors associated with means for these feedstuffs. Choline degradation (%) across incubation times averaged 57.0 for corn, 16.2 for corn gluten meal, 53.2 for alfalfa hay, and 37.8 for timothy hay. Because corn, corn gluten meal, alfalfa hay, and timothy hay are low in choline content, our assay method may not have been sensitive enough to detect precisely the change in their choline concentration with time of incubation. However, it is unlikely that feeding such low choline-containing feedstuffs to ruminants would make a significant contribution to the amount of postruminal flow of choline even if rumen degradation was not complete. For all other samples, initial loss of choline was rapid in the first 15 min, followed by a gradual increase, then a leveling off at 6 to 36 h.

Mean estimates for potentially degradable choline (%), rapidly degradable choline (%), and rate of choline degradation (h^{-1}), in vitro, are presented in Table 3. Estimates of potentially degradable choline were numerically higher for choline supplements but not different ($P>.05$) from cottonseed meal and soybean meal. Estimate of potentially degradable choline for fish meal and barley were lower ($P<.05$) than for synthetic choline supplements but not different ($P>.05$) from cottonseed meal and soybean meal. Rapidly degradable choline estimates were higher for choline supplements ($P<.05$) than for other sources (Table 3). Among feed sources, percentages of rapidly degradable choline in cottonseed meal, fish meal, and soybean meal were similar ($P>.05$). The estimate of rapidly degradable choline in barley was the lowest (55.3%) and different ($P<.05$) from all other feedstuffs and supplements. Rates of in vitro choline disappearance from various samples did not differ ($P>.05$) due to high variability associated with these means.

Mean estimates of effectively rumen degradable choline (%) calculated based on common outflow rate of .05/h are also in Table 3. Barley, cottonseed meal, fish meal, and soybean meal had similar ($P>.05$) estimates for rumen degradable choline. These estimates for choline stearate and choline chloride were 98.0 and 98.6, which were 13 to 18 percentage units higher ($P<.05$) than estimated rumen degradable choline in other feedstuffs (Table 3).

Neill et al. (12) suggested that choline of feedstuffs is rapidly liberated and extensively degraded in the rumen. Also, in the present experiment, even the lowest estimate of rumen-degradable choline associated with barley was 79.4%. From a short-term experiment, Atkins et al. (1) reported that 87.5% of the ruminally administered dose of choline (26 g) as choline chloride was degraded in the rumen. The results of the present experiment suggest that less than 2% of choline from each synthetic supplements would be predicted to escape rumen degradation.

Although barley, cottonseed meal, fish meal, and soybean meal had similar ($P>.05$) estimates for rumen-degradable choline, fish meal is far

TABLE 2. Mean (n = 4) choline disappearance (%) from samples at various times of incubation in vitro.

Sample	Time of incubation, h								
	.25	1	3	6	12	18	24	36	48
Corn silage									
$\bar{X}$	3.6	9.7	17.3	23.9	31.3	36.1	39.7	45.4	50.0
SE	3.62	5.12	9.53	12.64	14.71	14.53	13.77	12.96	13.63
Barley									
$\bar{X}$	57.4	63.5	74.1	78.0	79.3	82.2	84.0	84.3	85.6
SE	5.16	4.19	4.04	3.65	4.77	3.21	4.03	2.64	2.33
Corn									
$\bar{X}$	41.3	54.8	56.3	54.1	56.4	62.2	57.3	62.2	68.3
SE	12.63	7.40	11.55	25.34	8.58	5.76	4.62	5.32	3.20
Corn gluten meal									
$\bar{X}$	16.6	9.7	7.4	32.7	13.4	15.2	20.7	15.6	14.8
SE	15.81	5.13	1.61	.98	1.56	6.17	7.52	5.50	3.91
Cottonseed meal									
$\bar{X}$	77.8	79.2	83.1	85.8	82.4	83.3	84.9	87.5	89.7
SE	3.15	3.28	2.88	1.70	2.24	2.02	1.59	1.69	1.69
Fish meal									
$\bar{X}$	74.8	80.3	81.3	81.7	83.7	82.7	83.1	84.6	85.5
SE	1.37	1.53	1.94	1.93	1.97	1.83	.83	.82	1.74
Soybean meal									
$\bar{X}$	74.8	80.5	82.6	82.2	82.4	82.3	87.6	86.2	89.9
SE	2.27	2.99	3.52	3.28	1.79	1.30	1.68	.87	2.18
Alfalfa hay									
$\bar{X}$	22.9	31.5	49.4	43.4	53.8	70.5	78.7	56.8	71.5
SE	16.79	4.09	11.81	11.80	3.62	2.61	14.95	23.23	25.19
Timothy hay									
$\bar{X}$	23.0	43.8	50.6	51.0	38.3	28.5	25.1	32.1	47.6
SE	1.56	20.33	14.94	27.77	29.8	27.8	9.84	12.46	24.58
Choline stearate									
$\bar{X}$	92.3	96.3	96.5	99.4	96.8	96.9	96.8	96.9	98.6
SE	4.32	1.46	1.41	2.91	1.40	1.82	1.63	1.50	.28
Choline chloride									
$\bar{X}$	97.7	97.1	97.7	97.5	98.8	99.3	99.8	98.9	99.0
SE	.29	.11	.23	.31	.07	.34	.23	.35	.38

TABLE 3. Estimates of potentially degradable choline (P), rapidly degradable choline (R), rate of choline degradation (k) in vitro, and the effectively degradable choline in the rumen for selected samples.

Sample	P	R	k	Effective degradation
	(%)		(h^{-1})	(%)
Barley	84.5[a]	55.3[a]	.317	79.4[a]
Cottonseed meal	92.3[ab]	76.9[b]	.357	84.7[a]
Fish meal	84.2[a]	73.1[b]	.827	82.9[a]
Soybean meal	93.2[ab]	77.2[b]	.063	83.8[a]
Choline stearate	100[b]	95.8[c]	.304	98.0[b]
Choline chloride	98.2[b]	97.0[c]	.118	98.6[b]
SE	2.51	3.33	.163	1.55

[a,b,c]Means bearing different superscripts within a column differ ($P<.05$).

[1]Calculated based on an outflow rate of .05/h where effective degradation = R + (P − R) × k/(k + .05).

higher in choline content than other feedstuffs compared. It follows that feeding such feedstuffs high in choline content would increase the postruminal flow of choline and that using duodenal flow of choline as a marker to estimate the flow of rumen protozoa (8) may not be appropriate in cows fed choline-rich feedstuffs. However, a marginal increase in postruminally available choline realized by selecting feedstuffs high in choline content is of little importance with respect to the choline requirements of dairy cows. For example, if the postruminal choline requirement of a dairy cow was 30 g/d (6, 15), the cow would have to consume 39 kg/d of fish meal, which is neither practical nor economically feasible. Thus, in order to increase practically the postruminal availability of choline, synthetic sources containing much higher amounts of choline and protected from rumen degradation must be developed.

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