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IN VITRO RABBIT CECAL DIGESTIBILITY: EFFECT OF COPPER SULFATE AND DONOR AGE

A.O. Aderibigbe[1] and P.R. Cheeke[2]

[1] Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Oyo State, Nigeria

[2] OSU Rabbit Research Center, Oregon State University, Corvallis

Abstract

In vitro cecal digestibilities of high-, medium- and low-fiber substrates (straw, alfalfa meal, maize) were measured using cecal contents from rabbits of different ages (4, 8, 10 weeks of age, and adult). Dry matter digestibility of maize was lower ($P<0.05$) with 4 and 8 week-old rabbit cecal fluid than with cecal fluid from older animals. This supports the carbohydrate overload of the hindgut theory of enteritis.

There were no age differences in the digestibility of either alfalfa meal or straw. The addition of copper sulfate at concentrations of 50, 125, 250 and 500 ppm copper in the media markedly reduced digestibility of straw and alfalfa meal at all levels, indicating a pronounced sensitivity of cellulolytic organisms to copper. The digestibility of maize was strongly reduced at levels of 125 ppm or more, whereas the inhibition was less at 50 ppm copper. These results are relevant to use of copper sulfate as a feed additive for rabbits.

Introduction

Rabbit cecal contents can be used as the source of inocula in an in vitro digestibility system, as described by Aderibigbe et al. (1992a,b,c,d). The objectives of this experiment were (1) to determine if the in vitro digestibilities of three types of feedstuffs (high, medium and low fiber) were influenced by age of the donor animals, and (2) to determine if the addition of various levels of copper sulfate to the incubation flasks influenced in vitro digestibility. Both objectives are relevant to enteric disease in weanling rabbits, which has been suggested to involve carbohydrate overload of the hindgut of weanling rabbits (Cheeke and Patton, 1980). Copper sulfate levels up to 400 ppm in the diet reduce enteritis incidence (Patton et al., 1982). We hypothesize that the fermentative activity of high-starch ingredients such as maize might be lower in the cecum of the weanling rabbit than for older animals, contributing to an increased susceptibility to enteritis. We also hypothesize that copper sulfate, by its antibacterial properties, might reduce cecal fermentation.

Materials and Methods

Cecal fluids were collected from New Zealand White rabbits. The animals were fed a diet with 54% alfalfa meal, 26% wheat mill run and 15% soybean meal as the major ingredients. Animals in each age group were killed and the cecums obtained, weighed and cecal contents quickly placed in an anaerobic (CO_2) container. A mixture of one part cecal contents, two parts nutrient buffer (Goering and Van Soest, 1970) and two parts distilled water was prepared. All solutions had been gassed with CO_2, covered and kept at 39° C. The mixture was thoroughly mixed, filtered through two layers of cheesecloth and left at 39° C for one hour in a separatory funnel. Fine feed particles in the bottom layer were removed and the remaining portion filtered through two layers of cheesecloth. The solution was mixed with nutrient buffer and distilled water in a ratio of 2:1:1, respectively. Fifty ml of the mixture was used as the inoculum for each in vitro cecal fermentation.

Feed ingredients used in the in vitro trials were ground through a 1 mm screen in a Wiley mill. Digestibility values were statistically analyzed by two-way analysis of variance, with means compared using Duncan's multiple range test (Steel and Torrie, 1980).

Trial 1

The objective was to observe the effect of donor rabbit age on cecal digestibility values using feedstuffs varying in fiber and soluble carbohydrate contents. The substrates consisted of annual ryegrass straw, alfalfa meal and yellow corn grain (Table 1). Four groups of rabbits of different ages (4, 8, 10 weeks and adult) served as sources of cecal fluid. The average total weights of cecum plus contents were 57 g, 90 g, 132 g and 200 g, respectively, for 4, 8, 10 weeks and adult rabbits. About 80% of the total weight consisted of the contents. In vitro incubations with rabbit cecal fluid were conducted for 48 hours, using 5 samples (1 g substrate) per ingredient for each age group.

Trial 2

The effect of various levels of copper sulfate on digestibility with rabbit cecal fluid was tested, using annual ryegrass straw, alfalfa meal and yellow corn grain as substrates. Five levels of copper sulfate were added to the final volume of inoculum to supply 0, 50, 125, 250 or 500 ppm (w/w) of copper, respectively. Cecal fluid was collected from 12 week old rabbits. In vitro incubations were for 48 hours with 5 flasks per sample.

Results and Discussion

The in vitro digestibility of yellow maize (corn) in cecal fluid of 4 and 8 week old rabbits was significantly lower ($P<0.05$) than for the 10 week old and adult animals

(Table 2). This suggests that the cecal microbes of the young post-weaning rabbit are limited in their ability to digest starch. Along with the low digestibility of starch in the small intestine of the young rabbit (Cheeke, 1987), this observation would suggest that an excess of poorly digested maize carbohydrate could accumulate in the cecum and serve as a substrate for pathogens such as E. coli and Clostridium spiroforme. The results of this trial support the carbohydrate overload hypothesis of Cheeke and Patton (1980). Robinson et al. (1988) found that starter and grower diets containing 30% maize and 30% barley provoked a very high incidence of enteritis mortality (about 40%). Thus, the totality of these studies suggest a sensitivity of young rabbits to carbohydrate overload, leading to cecal dysbiosis and diarrhea.

Table 1. Chemical composition of the feed ingredients used as substrates for in vitro cecal digestion studies.

Item	Ingredient		
	Annual Ryegrass Straw	Alfalfa Meal	Maize (Yellow) Dent)
Dry matter (DM, %)	90.6	92.2	90.1
Organic matter (OM, %)	84.9	83.1	88.8
Analyses, % DM:			
Crude protein (CP)	5.8	18.7	9.5
Acid detergent fiber (ADF)	47.0	34.1	3.2
Cell contents (CC)	27.2	55.7	85.0
Ash	5.7	9.1	1.3

There were no major age effects in digestibility of ryegrass straw and alfalfa meal, although the dry matter digestibility of alfalfa meal in the older rabbits was significantly ($P<0.05$) higher (Table 2). The data do not suggest any substantial improvement in cecal digestion of fibrous ingredients with older rabbits. Similar trends were observed with organic matter digestibilities (Table 3).

The addition of copper sulfate to the media had a pronounced depressing effect on dry matter (Table 4) and organic matter (Table 5) digestibilities. The depression was most pronounced with the ryegrass straw as the substrate. At higher levels of copper sulfate, digestibility of straw and maize was almost completely eliminated, whereas with alfalfa meal, about 15% digestibility was observed. One possibility to account for this is

Table 2. In vitro cecal dry matter digestibility of three substrates by cecal contents from rabbits of different ages.

	Age of Rabbit			
Substrate	4 weeks	8 weeks	10 weeks	Adult
Ryegrass straw	$15.4^{b,d}$	$7.9^{a,d}$	$13.1^{b,d}$	$13.8^{b,d}$
Alfalfa meal	$26.2^{a,e}$	$23.7^{a,e}$	$29.3^{b,e}$	$33.7^{c,e}$
Yellow maize	$60.5^{a,f}$	$58.9^{a,f}$	$71.3^{b,f}$	$75.1^{c,f}$

^{a,b,c} Means with different superscripts in each row differ ($P<0.05$).

^{d,e,f} Means with different superscripts in each column differ ($P<0.05$).

that the apparent digestibility of alfalfa is an artifact, and is due simply to solubilization of about 15% of the alfalfa dry matter in the medium. This could be tested in further work by autoclaving the cecal contents to have a negative control. Another possibility is that alfalfa may contain substances that inactivate or bind copper. Grobner et al. (1986) found that the toxicity of high dietary copper levels to rabbits was markedly reduced with diets high in alfalfa.

Table 3. In vitro cecal organic matter digestibility of three substrates by cecal contents from rabbits of different ages.

	Age of Rabbit			
Substrate	4 weeks	8 weeks	10 weeks	Adult
Ryegrass straw	$17.6^{c,d}$	$10.2^{a,d}$	$15.6^{b,c,d}$	$14.1^{b,d}$
Alfalfa meal	$31.2^{b,e}$	$27.6^{a,e}$	$34.7^{c,e}$	$35.6^{c,e}$
Yellow maize	$63.8^{a,f}$	$61.7^{a,f}$	$72.9^{b,f}$	$76.6^{c,f}$

^{a,b,c} Means with different superscripts in each row differ ($P<0.05$).

^{d,e,f} Means with different superscripts in each column differ ($P<0.05$).

Table 4. In vitro cecal dry matter digestibility of three substrates by cecal contents with various levels of copper added to the media.

	Level of Copper (ppm)				
Substrate	0	50	125	250	500
Ryegrass straw	$31.4^{c,e}$	$6.0^{b,e}$	$2.0^{a,e}$	$1.5^{a,e}$	$1.1^{a,e}$
Alfalfa meal	$43.7^{c,f}$	$19.1^{b,f}$	$15.8^{a,g}$	$14.0^{a,f}$	$15.0^{a,f}$
Yellow maize	$71.1^{d,g}$	$59.8^{c,g}$	$8.2^{b,f}$	$1.3^{a,e}$	$3.3^{a,e}$

^{a,b,c,d} Means with different superscripts in each row differ ($P<0.05$).

^{e,f,g} Means with different superscripts in each column differ ($P<0.05$).

Table 5. In vitro cecal organic matter digestibility of three substrates by cecal contents with various levels of copper added to the media.

	Level of Copper (ppm)				
Substrate	0	50	125	250	500
Ryegrass straw	$34.7^{c,e}$	$7.7^{b,e}$	$3.6^{a,e}$	$2.2^{a,e}$	$3.6^{a,e}$
Alfalfa meal	$45.9^{d,f}$	$22.1^{c,f}$	$19.0^{b,g}$	$15.6^{a,f}$	$16.6^{a,g}$
Yellow maize	$72.9^{d,g}$	$63.1^{c,g}$	$11.6^{b,f}$	$5.0^{a,e}$	$6.7^{a,f}$

^{a,b,c,d} Means with different superscripts in each row differ ($P<0.05$).

^{c,f,g} Means with different superscripts in each column differ ($P<0.05$).

The sensitivity of cecal microbes to high copper suggests that caution should be used when copper sulfate is employed as a feed additive. Further studies are suggested, involving the determination of cecal copper concentrations when different dietary levels of copper are fed.

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