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IN VITRO RUMEN AND RABBIT CECUM DIGESTIBILITIES: I. EFFECTS OF PROBIOTICS AND INCUBATION TIME

A.O. Aderibigbe[1], P.R. Cheeke[2], and N.M. Patton[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 rumen and cecal digestion studies were conducted on annual ryegrass straw, alfalfa meal and corn, supplemented with or without probiotic (Yea-Sacc or Lacto-Sacc; 2 mg/g DM). Rumen fluids for the in vitro incubations were obtained from two rumen-fistulated crossbred cows while cecal fluids were obtained from the ceca of adult rabbits (> 12 weeks old). Closed in vitro incubations were conducted on triplicate samples for 12, 24 or 48 h. In vitro rumen and cecal digestibilities of the different substrates increased with increasing periods of incubation, and were in the order: corn > alfalfa meal > annual ryegrass straw. In general, there were no effects of probiotics on in vitro rumen or cecal digestibility of the different substrates. Digestibility of the substrates was higher with rumen than with cecal inoculum. There was a large increase in cecal digestibility of the substrates from 24 to 48 h, indicating the need for sufficient period for adequate cecal microbial digestion. Rabbit cecal fluid was more effective in digesting corn (amylolytic digestion) than alfalfa and straw (cellulolytic digestion), but was inferior to rumen digestion with all substrates. There was low relative cecal digestibility of fiber as compared to digestibility with rumen fluid. In vitro cecal digestion studies could serve as a means of preliminary evaluation of the nutritive value of feedstuffs for rabbits. There is a need for more research studies in the area of the relationship between in vitro cecal digestibility and in vivo digestibility of various rabbit diets.

Introduction

In vitro rumen fermentation studies have been used for several decades to estimate digestibilities of forages and feed samples without animal digestion trials (Shelter, 1962; Tilley and Terry, 1963; Goering and Van Soest, 1970; Maertens et al., 1971; Schneider and Flatt, 1975; Aderibigbe, 1980). Schneider and Flatt (1975) pointed out that in vitro procedures are usually more reliable when applied to forages within species, within grasses or within legumes. No report is available on in vitro rabbit cecal fermentation studies. The objectives in the research reported herein were (a) to compare the rate of in vitro digestibilities of annual ryegrass straw alfalfa meal or corn in the rumen of cattle with that in the ceca of rabbits and (b) to observe the effects of probiotics (Yea-Sacc or

Lacto-Sacc) and incubation periods on the rate of in vitro fiber and starch digestion in the rumen of cattle and the ceca of rabbits.

Materials and Methods

Annual ryegrass straw, alfalfa meal and yellow dent corn were each ground in a Wiley mill (1 mm screen) and kept in covered plastic containers. Triplicate samples (1 g DM basis) of each ingredient were supplemented with or without probiotics (Yea-Sacc or Lacto-Sacc; 2 mg/g DM) and used as substrates for in vitro rumen or cecal fermentations. Rumen fluids for the in vitro rumen incubations were obtained from two rumen-fistulated crossbred cows maintained on grass pasture and a corn containing concentrate for 2 weeks before the first collection in order to adapt the rumen microbes. rumen fluids were collected 2 h after the morning feeding and filtered through two layers of cheesecloth into a pre-warmed thermos bottle (39° C). Closed in vitro rumen incubations were conducted for 12, 24 or 48 h, respectively, by the method described by Goering and Van Soest (1970). Inoculum consisted of 50 ml of a mixture of one part rumen liquor, two parts nutrient buffer solution and two parts distilled water.

Cecal fluids for the in vitro cecal incubations consisted of one part cecal content of adult rabbits (>12 weeks old) which had been maintained on a standard Oregon State University rabbit diet (OSU 48[1]) for 2 weeks before slaughter and collection of their cecal contents, two parts of a nutrient buffer solution (Goering and Van Soest, 1970) and two parts distilled water. The nutrient buffer solution and distilled water had previously been gassed with CO_2, covered and kept at 39° C before addition of the cecal contents. The components were thoroughly mixed together on a hot plate (39° C) using a stirring rod and magnetic stirrer (10 min), filtered through two layers of cheesecloth into a pre-warmed separatory funnel (39° C) and kept in an incubator at 39° C for 1 hr. in order to separate the feed particles from the microbial portion. The feed particles in the bottom layer were drained out and the remaining portion was filtered through two layers of cheesecloth into a pre-warmed measuring cylinder (39° C). The solution was mixed with prewarmed (39° C) and pregassed (CO_2) nutrient buffer solution and distilled water at a ratio of 2:1:1, respectively. Fifty ml of the mixture served as the inoculum for each of the in vitro cecal fermentations. Closed in vitro cecal incubations were conducted for 12, 24 or 48 h, respectively. Data obtained for in vitro rumen and cecal dry matter digestibilities (IVRDMD and IVCDMD, respectively) and organic matter digestibilities (IVROMD and IVCOMD, respectively) were analyzed by use of a two-way analysis of variance as described by Neter and Wasserman (1977). Means were compared using Duncan's Multiple Range test as outlined by Steel and Torrie (1980).

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[1] OSU 48 contained 54% alfalfa meal, 26% wheat mill run, 15% soybean meal, 3% molasses, 1.25% corn, 0.5% trace mineralized salt, 0.25% dicalcium phosphate, 7 million IU vitamin A and 20,000 IU vitamin E.

Results and Discussion

The results of the % in vitro rumen dry matter (IVRDMD) and organic matter (IVROMD) digestibilities of the various substrates with or without probiotics are shown in Tables 1 and 2, respectively. The % IVRDMD and IVROMD for each substrate increased with increasing periods of incubation ($P < .05$) and the prominence of this effect ranged in the order of annual ryegrass straw > alfalfa meal > corn. This indicated the need for a sufficient period for adequate microbial activity in digesting the low quality substrates. The % IVRDMD and IVROMD for the different substrates during each period of incubation followed the trend corn > alfalfa meal > annual ryegrass straw ($P < .05$), reflecting the quality of the substrates. There was no significant effect ($P < .05$) of probiotic supplementation on IVRDMD or IVROMD among either the various substrates or during each period of incubation.

The % in vitro cecal dry matter (IVCDMD) and organic matter (IVCOMD) digestibilities of the various substrates are shown in Tables 3 and 4, respectively. IVCDMD and IVCOMD followed similar trends as those described for IVRDMD and IVROMD. However, a significant effect ($P < .05$) of probiotics on IVCDMD was observed for alfalfa meal during 24 h incubation which followed the trend Lacto-Sacc > no probiotic = Yea-Sacc. There was a large increase in cecal digestibility of the substrates from 24 to 48 h, indicating the need for a sufficient period for adequate cecal microbial digestion of the substrates. The results of the comparisons between in vitro rumen and in vitro cecal digestibilities of dry matter and organic matter of the different substrates with or without probiotics are shown in Tables 5 and 6, respectively. IVRDMD values were higher ($P < .05$) than those of IVCDMD for each of the substrates and during each incubation period. IVROMD values were similarly higher than those of IVCOMD values. Thus, in vitro microbial digestion of the substrates was higher with rumen inoculum than with cecal inoculum at the applied concentrations. Tables 7 and 8 show the results of IVCDMD and IVCOMD as percentages of IVRDMD and IVROMD, respectively. The relative cecal digestibilities of the substrates increased with increasing period of incubation. Among the different substrates, the trend was in the order: corn > alfalfa meal > straw, irrespective of incubation period. The relative IVCDMD (Table 7) were generally lower than the relative IVCOMD (Table 8).

Conclusion

In vitro cecal digestion studies could serve as a means of preliminary evaluation of the nutritive value of feedstuffs for rabbits. There were no effects of probiotics (Yea-Sacc or Lacto-Sacc) on in vitro rumen or cecal digestibility of the different substrates. There is a need for more research studies in the area of the relationship between in vitro cecal digestibility and in vivo digestibility of various rabbit diets. The ultimate goal should include predicting in vivo utilization of the various diets from in vitro studies.

Table 1. In vitro rumen dry matter digestibility (IVRDMD, %) of annual ryegrass straw, alfalfa meal or corn supplemented with or without probiotics (Yea-Sacc or Lacto-Sacc) and incubated for different periods (12, 24 or 48 h).

	Annual Ryegrass Straw			Alfalfa Meal			Corn		
Probiotics	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
None	$24.3^{a,d}$	$36.8^{b,g}$	$54.4^{c,j}$	$40.6^{a,e}$	$49.9^{b,h}$	$63.4^{c,k}$	$63.8^{a,f}$	$74.7^{b,i}$	$83.2^{c,i}$
Yea-Sacc	$23.6^{a,d}$	$36.3^{b,g}$	$53.5^{c,j}$	$41.4^{a,e}$	$50.3^{b,h}$	$63.3^{c,k}$	$66.2^{a,f}$	$74.5^{b,i}$	$83.2^{c,i}$
Lacto-Sacc	$24.6^{a,d}$	$37.0^{b,g}$	$52.5^{c,j}$	$42.3^{a,e}$	$51.5^{b,h}$	$65.5^{c,k}$	$64.8^{a,f}$	$74.1^{b,i}$	$82.8^{c,i}$

^{a,b,c} Means with a different superscript in each row for each substrate (straw, alfalfa meal or corn) differ ($P<.05$).

^{d,e,f} Means with a different superscript in each row for 12 h differ ($P<.05$).

^{g,h,i} Means with a different superscript in each row for 24 h differ ($P<.05$).

^{j,k,l} Means with a different superscript in each row for 48 h differ ($P<.05$).

Table 2. In vitro rumen organic matter digestibility (IVROMD, %) of annual ryegrass straw, alfalfa meal or corn supplemented with or without probiotics (Yea-Sacc or Lacto-Sacc) and incubated for different periods (12, 24 or 48 h).

	Annual Ryegrass Straw			Alfalfa Meal			Corn		
Probiotics	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
None	$23.6^{a,d}$	$34.4^{b,g}$	$50.6^{c,j}$	$37.6^{a,e}$	$46.5^{b,h}$	$59.2^{c,k}$	$57.5^{a,f}$	$67.5^{b,i}$	$74.1^{c,i}$
Yea-Sacc	$22.9^{a,d}$	$34.1^{b,g}$	$49.5^{c,j}$	$38.5^{a,e}$	$46.9^{b,h}$	$59.2^{c,k}$	$59.6^{a,f}$	$67.8^{b,i}$	$74.7^{c,i}$
Lacto-Sacc	$23.4^{a,d}$	$34.1^{b,g}$	$48.2^{c,j}$	$38.8^{a,e}$	$47.7^{b,h}$	$60.7^{c,k}$	$58.6^{a,f}$	$67.2^{b,i}$	$74.3^{c,i}$

^{a,b,c} Means with a different superscript in each row for each substrate (straw, alfalfa meal or corn) differ ($P < .05$).

^{d,e,f} Means with a different superscript in each row for 12 h differ ($P < .05$).

^{g,h,i} Means with a different superscript in each row for 24 h differ ($P < .05$).

^{j,k,l} Means with a different superscript in each row for 48 h differ ($P < .05$).

Table 3. In vitro cecal dry matter digestibility (IVCDMD, %) of annual ryegrass straw, alfalfa meal or corn supplemented with or without probiotics (Yea-Sacc or Lacto-Sacc) and incubated for different periods (12, 24 or 48 h).

	Annual Ryegrass Straw			Alfalfa Meal			Corn		
Probiotics	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
None	$7.0^{a,d}$	$11.8^{b,g}$	$31.8^{c,j}$	$25.4^{a,e}$	$28.8^{b,h,m}$	$44.3^{c,k}$	$41.2^{a,f}$	$58.6^{b,i}$	$73.7^{c,i}$
Yea-Sacc	$6.7^{a,d}$	$10.7^{b,g}$	$32.4^{c,j}$	$26.8^{a,e}$	$30.7^{b,h,m,n}$	$43.4^{c,k}$	$39.0^{a,f}$	$60.8^{b,i}$	$72.6^{c,i}$
Lacto-Sacc	$8.5^{a,d}$	$12.0^{b,g}$	$31.8^{c,j}$	$27.0^{a,e}$	$32.7^{b,h,n}$	$44.7^{c,k}$	$39.4^{a,f}$	$59.8^{b,i}$	$74.7^{c,i}$

^{a,b,c} Means with a different superscript in each row for each substrate (straw, alfalfa meal or corn) differ ($P < .05$).

^{d,e,f} Means with a different superscript in each row for 12 h differ ($P < .05$).

^{g,h,i} Means with a different superscript in each row for 24 h differ ($P < .05$).

^{j,k,l} Means with a different superscript in each row for 48 h differ ($P < .05$).

^{m,n} Means with a different superscript in each column differ ($P < .05$).

Table 4. In vitro cecal organic matter digestibility (IVCOMD, %) of annual ryegrass straw, alfalfa meal or corn supplemented with or without probiotics (Yea-Sacc or Lacto-Sacc) and incubated for different periods (12, 24, or 48 h).

	Annual Ryegrass Straw			Alfalfa Meal			Corn		
Probiotics	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
None	$8.6^{a,d}$	$13.6^{b,g}$	$31.7^{c,j}$	$24.2^{a,e}$	$28.1^{b,h}$	$42.8^{c,k}$	$39.7^{a,f}$	$52.8^{b,i}$	$66.2^{c,i}$
Yea-Sacc	$8.3^{a,d}$	$12.7^{b,g}$	$32.3^{c,j}$	$26.0^{a,e}$	$29.6^{b,h}$	$42.2^{c,k}$	$37.2^{a,f}$	$54.9^{b,i}$	$65.7^{c,i}$
Lacto-Sacc	$9.2^{a,d}$	$13.5^{b,g}$	$31.9^{c,j}$	$26.0^{a,e}$	$31.1^{b,h}$	$43.3^{c,k}$	$36.9^{a,f}$	$53.7^{b,i}$	$67.3^{c,i}$

^{a,b,c} Means with a different superscript in each row for each substrate (straw, alfalfa meal or corn) differ ($P<.05$).

^{d,e,f} Means with a different superscript in each row for 12 h differ ($P<.05$).

^{g,h,i} Means with a different superscript in each row for 24 h differ ($P<.05$).

^{j,k,l} Means with a different superscript in each row for 48 h differ ($P<.05$).

Table 5. Comparison between in vitro rumen and in vitro cecal dry matter digestibility (%) of annual ryegrass straw, alfalfa meal or corn supplemented with or without probiotics (Yea-Sacc or Lacto-Sacc) and incubated for different periods (12, 24 or 48 h).

	12 h		24 h		48 h	
	Rumen	Cecum	Rumen	Cecum	Rumen	Cecum
Straw	24.3[b]	7.0[a]	36.8[b]	11.8[a]	54.4[b]	31.8[a]
Straw plus Yea-Sacc	23.6[b]	6.7[a]	36.3[b]	10.7[a]	53.5[b]	32.4[a]
Straw plus Lacto-Sacc	24.6[b]	8.5[a]	37.0[b]	12.0[a]	52.5[b]	31.8[a]
Alfalfa meal	40.6[b]	25.4[a]	49.9[b]	28.8[a]	63.4[b]	44.3[a]
Alfalfa meal plus Yea-Sacc	41.4[b]	26.8[a]	50.3[b]	30.7[a]	63.3[b]	43.4[a]
Alfalfa meal plus Lacto-Sacc	42.3[b]	27.0[a]	51.5[b]	32.7[a]	65.5[b]	44.7[a]
Corn	63.8[b]	41.2[a]	74.7[b]	58.6[a]	83.2[b]	73.7[a]
Corn plus Yea-Sacc	66.2[b]	39.0[a]	74.5[b]	60.8[a]	83.2[b]	72.6[a]
Corn plus Lacto-Sacc	64.8[b]	39.4[a]	74.1[b]	59.8[a]	82.8[b]	74.7[a]

[a,b] Means with a different superscript in each row for each hour (12, 24 or 48 h) differ ($P < .05$).

Table 6. Comparison between in vitro rumen and in vitro cecal colonic organic matter digestibilities (%) of annual ryegrass straw, alfalfa meal or corn supplemented with or without probiotics (Yea-Sacc or Lacto-Sacc) and incubated for different periods (12, 24 or 48 h).

	12 h		24 h		48 h	
	Rumen	Cecum	Rumen	Cecum	Rumen	Cecum
Straw	23.6[b]	8.6[a]	34.4[b]	13.6[a]	50.6[b]	31.7[a]
Straw plus Yea-Sacc	22.9[b]	8.3[a]	34.1[b]	12.7[a]	49.5[b]	32.3[a]
Straw plus Lacto-Sacc	23.4[b]	9.2[a]	34.1[b]	13.5[a]	48.2[b]	31.9[a]
Alfalfa meal	37.6[b]	24.2[a]	46.5[b]	28.1[a]	59.2[b]	42.8[a]
Alfalfa meal plus Yea-Sacc	38.5[b]	26.0[a]	46.9[b]	29.6[a]	59.2[b]	42.2[a]
Alfalfa meal plus Lacto-Sacc	38.8[b]	26.0[a]	47.7[b]	31.1[a]	60.7[b]	43.3[a]
Corn	57.5[b]	39.7[a]	67.5[b]	52.8[a]	74.1[b]	66.2[a]
Corn plus Yea-Sacc	59.6[b]	37.2[a]	67.8[b]	54.9[a]	74.7[b]	65.7[a]
Corn plus Lacto-Sacc	58.6[b]	36.9[a]	67.2[b]	53.7[a]	74.3[b]	67.3[a]

[a,b] Means with a different superscript in each row for each hour (12, 24 or 48 h) differ ($P < .05$).

Table 7. In vitro cecal dry matter digestibility as percentage of in vitro rumen dry matter digestibility for annual ryegrass straw, alfalfa meal or corn at different incubation periods (12, 24 or 48 h).

	Period of In Vitro Incubation		
Substrate	12 h	24 h	48 h
Straw	28.8	32.1	58.5
Alfalfa meal	62.6	57.7	69.9
Corn	64.6	78.4	88.6

Table 8. In vitro cecal organic matter digestibility as percentage of in vitro rumen organic matter digestibility for annual ryegrass straw, alfalfa meal or corn at different incubation periods (12, 24 or 48 h).

	Period of In Vitro Incubation		
Substrate	12 h	24 h	48 h
Straw	36.4	39.5	62.6
Alfalfa meal	64.4	60.4	72.3
Corn	69.0	78.2	89.3

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