

**IN VITRO RUMEN AND RABBIT CECUM DIGESTIBILITIES:**  
**II. EFFECTS OF EXTENDED INCUBATION TIME**

A.O. Aderibigbe<sup>1</sup>, P.R. Cheeke<sup>2</sup>, and N.M. Patton<sup>2</sup>

<sup>1</sup> Department of Animal Science, Obafemi Awolowo University,  
Ile-Ife, Oyo State, Nigeria

<sup>2</sup> OSU Rabbit Research Center, Oregon State University, Corvallis

**Abstract**

In vitro rumen and cecal digestion studies were conducted on pentuplicate samples of annual ryegrass straw, alfalfa meal and corn for 24, 48, 72 or 96 h using the methods described by Aderibigbe *et al.* (1992). Rumen and cecal digestibilities of the substrates generally increased with increasing periods of incubation up to 72 h and were in the order: corn > alfalfa meal > annual ryegrass straw. The optimum incubation periods for in vitro rumen and cecal digestibilities of the substrates were 48 and 72 h, respectively. Digestibility of the substrates was higher with rumen than with cecal inoculum. Cecal digestibility as percentage of rumen digestibility for annual ryegrass straw increased with increasing periods of incubation up to 72 h while those for alfalfa meal and corn increased with increasing periods of incubation up to 96 h. The highest increase for annual ryegrass straw and alfalfa meal occurred between 48 and 72 h, while that for corn occurred between 24 and 48 h. There is a need for research on the effect of age of rabbits on the digestibility potentials of inoculum collected from the animals.

**Introduction**

In vitro cecal fermentation is currently receiving attention as a means of preliminary evaluation of the nutritive value of feedstuffs and feed samples for rabbits. Aderibigbe *et al.* (1992) conducted in vitro cecal and rumen fermentation studies on some feed ingredients with incubation periods of up to 48 h and observed (a) higher digestibility values for rumen fermentation than for cecal fermentation, (b) an increased digestibility of the various substrates with increasing periods of incubation and (c) a large increase in digestibility of the substrates from 24 to 48 h. They pointed out the need for sufficient period of incubation for adequate cecal digestion of the substrates. The objective of this study was to observe the effects of extended periods of incubation on in vitro rabbit cecal or cattle rumen fermentation of annual ryegrass straw, alfalfa meal or corn.

**Materials and Methods**

In vitro rabbit cecal and cattle rumen fermentations were conducted on ground (Wiley mill; 1 mm screen) annual ryegrass straw, alfalfa meal and yellow dent corn using the

methods described by Aderibigbe *et al.* (1992). Closed *in vitro* incubations were conducted for 24, 48, 72 or 96 h using pentuplicate samples (1 g DM basis) of each ingredient. The percent *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).

### Results and Discussion

The percentage *in vitro* rumen dry matter (IVRDMD) and organic matter (IVROMD) digestibilities of the various substrates incubated for the different periods are shown in Tables 1 and 2, respectively. IVRDMD for the substrates (Table 1) generally increased with increasing period of incubation up to 72 h ( $P < .05$ ) with the largest increase occurring between 24 and 48 h. IVRDMD for the substrates during each period of incubation generally ranged in the order: corn > alfalfa meal > annual ryegrass straw ( $P < .05$ ). IVROMD for annual ryegrass straw (Table 2) also increased with increasing periods of incubation up to 72 h ( $P < .05$ ) while that of alfalfa meal increased with increasing period of incubation up to 48 h ( $P < .05$ ). IVROMD for corn was in the order: 96 h = 72 h > 48 h = 24 h ( $P < .05$ ). IVROMD for the substrates during each period of incubation also ranged in the order: corn > alfalfa meal > annual ryegrass straw ( $P < .05$ ).

Table 1. *In vitro* rumen dry matter digestibility (IVRDMD, %) of annual ryegrass straw, alfalfa meal or corn incubated for different periods (24, 48, 72 or 96 h).

Substrate	Period of Incubation			
	24 h	48 h	72 h	96 h
Annual ryegrass straw	31.1 <sup>a,d</sup>	43.0 <sup>b,d</sup>	48.2 <sup>c,d</sup>	50.7 <sup>c,d</sup>
Alfalfa meal	43.2 <sup>a,c</sup>	51.7 <sup>b,c</sup>	53.4 <sup>b,c</sup>	52.0 <sup>b,d</sup>
Corn	68.6 <sup>a,f</sup>	73.3 <sup>b,f</sup>	75.8 <sup>b,c,f</sup>	77.2 <sup>c,e</sup>

<sup>a,b,c</sup> Means with a different superscript in each row differ ( $P < .05$ ).

<sup>d,e,f</sup> Means with a different superscript in each column differ ( $P < .05$ ).

The results of *in vitro* cecal dry matter (IVCDMD) and organic matter (IVCOMD) digestibilities of the various substrates incubated for the different periods are shown on Tables 3 and 4, respectively. IVCDMD and IVCOMD for the substrates increased with increasing periods of incubation up to 72 h ( $P < .05$ ). The largest increase for annual

ryegrass straw and alfalfa meal occurred between 48 and 72 h, while that for corn occurred between 24 and 48 h. IVCDMD and IVCOMD for the substrates during each period of incubation also ranged in the order: corn > alfalfa meal > annual ryegrass straw ( $P < .05$ ). Thus, 48 h seemed to be the optimum incubation period for in vitro rumen digestibility, while 72 h seemed to be the optimum period for in vitro cecal incubation at the applied concentrations of inoculum. It should be pointed out that IVCDMD and IVCOMD for 24 and 48 h were lower than those reported by Aderibigbe *et al.* (1991) for the respective periods. This was probably due to (a) variability in digestibility between batches of in vitro incubation and (b) different sources of cecal inoculum for the different studies. Although the cecal inoculum for the two studies were collected from adult rabbits (> 12 weeks old), those for the earlier study were collected from culled does and bucks, while those for the latter study were collected from young fryer rabbits. Thus, there would seem to be an effect of age of rabbits on the digestibility potentials of inoculum from the animals.

Table 2. In vitro rumen organic matter digestibility (IVROMD, %) of annual ryegrass straw, alfalfa meal or corn incubated for different periods (24, 48, 72 or 96 h).

Substrate	Period of Incubation			
	24 h	48 h	72 h	96 h
Annual ryegrass straw	34.0 <sup>a,d</sup>	44.7 <sup>b,d</sup>	50.2 <sup>c,d</sup>	52.3 <sup>c,d</sup>
Alfalfa meal	46.3 <sup>a,c</sup>	55.7 <sup>b,c</sup>	57.5 <sup>b,e</sup>	56.0 <sup>b,e</sup>
Corn	72.6 <sup>a,f</sup>	74.7 <sup>a,f</sup>	78.6 <sup>b,f</sup>	78.6 <sup>b,f</sup>

<sup>a,b,c</sup> Means with a different superscript in each row differ ( $P < .05$ ).

<sup>d,e,f</sup> Means with a different superscript in each column differ ( $P < .05$ ).

Tables 5 and 6 show results of comparisons between in vitro rumen and in vitro cecal digestibilities of dry matter and organic matter of the different substrates, respectively. IVRDMD values were higher ( $P < .05$ ) than IVCDMD values for each incubation period. IVROMD values were similarly higher than IVCOMD values. These agreed with the findings of Aderibigbe *et al.* (1992) who observed that in vitro microbial digestion of the various substrates were higher with rumen inoculum than with cecal inoculum at the applied concentrations. The results of IVCDMD and IVCOMD as percentages of IVRDMD and IVROMD, respectively, are shown in Tables 7 and 8. The relative cecal dry matter and organic matter digestibilities of annual ryegrass straw increased with increasing periods of incubation up to 72 h while those of alfalfa meal increased with increasing periods of incubation up to 96 h, with the highest increase for both substrates occurring between 48 and 72 h. The relative cecal dry matter and organic matter

digestibilities of corn also increased with increasing period of incubation up to 96 h. However, the highest increase occurred between 24 and 48 h while the lowest increase occurred between 48 and 72 h. Trends for the relative cecal dry matter and organic matter digestibilities of the substrates were in the order: corn > alfalfa meal > straw, irrespective of incubation period. It should be noted that the relative cecal dry matter and organic matter digestibilities of the substrates during 24 and 48 h incubation periods were lower than those reported by Aderibigbe *et al.* (1992). This was due to lower IVCDMD and IVCOMD values obtained for the present study which had been explained previously. The relative IVCDMD (Table 7) were generally lower than the relative IVCOMD (Table 8).

Table 3. *In vitro* cecal dry matter digestibility (IVCDMD, %) of annual ryegrass straw, alfalfa meal or corn incubated for different periods (24, 48, 72 or 96 h).

Substrate	Period of Incubation			
	24 h	48 h	72 h	96 h
Annual ryegrass straw	6.7 <sup>a,c</sup>	13.5 <sup>b,c</sup>	28.0 <sup>c,e</sup>	29.7 <sup>c,e</sup>
Alfalfa meal	20.2 <sup>a,f</sup>	25.9 <sup>b,f</sup>	33.5 <sup>c,f</sup>	34.3 <sup>c,f</sup>
Corn	35.0 <sup>a,g</sup>	55.2 <sup>b,g</sup>	59.0 <sup>c,g</sup>	63.0 <sup>d,g</sup>

<sup>a,b,c,d</sup> Means with a different superscript in each row differ ( $P < .05$ )

<sup>e,f,g</sup> 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 incubated for different periods (24, 48 72 or 96 h).

Substrate	Period of Incubation			
	24 h	48 h	72 h	96 h
Annual ryegrass straw	9.7 <sup>a,c</sup>	16.7 <sup>b,c</sup>	32.0 <sup>c,e</sup>	33.7 <sup>c,e</sup>
Alfalfa meal	24.3 <sup>a,f</sup>	31.0 <sup>b,f</sup>	40.0 <sup>c,f</sup>	40.6 <sup>c,f</sup>
Corn	37.8 <sup>a,g</sup>	57.3 <sup>b,g</sup>	61.1 <sup>c,g</sup>	65.5 <sup>d,g</sup>

<sup>a,b,c,d</sup> Means with a different superscript in each row differ ( $P < .05$ ).

<sup>e,f,g</sup> Means with a different superscript in each column differ ( $P < .05$ ).

Table 5. In vitro rumen versus cecal dry matter digestibility of ryegrass straw, alfalfa meal and corn incubated for different periods (24, 48, 72 or 99 h).

Substrate	Period of Incubation							
	24 h		48 h		72 h		96 h	
	IVRDMD <sup>a</sup>	IVCDMD <sup>b</sup>	IVRDMD <sup>a</sup>	IVCDMD <sup>b</sup>	IVRDMD <sup>a</sup>	IVCDMD <sup>b</sup>	IVRDMD <sup>a</sup>	IVCDMD <sup>b</sup>
Annual ryegrass straw	31.1 <sup>d,g</sup>	6.7 <sup>c,g</sup>	43.0 <sup>d,g</sup>	13.5 <sup>c,g</sup>	48.2 <sup>d,g</sup>	28.0 <sup>c,g</sup>	50.7 <sup>d,g</sup>	29.7 <sup>c,g</sup>
Alfalfa meal	43.2 <sup>d,f</sup>	20.2 <sup>c,f</sup>	51.7 <sup>d,f</sup>	25.9 <sup>c,f</sup>	53.4 <sup>d,f</sup>	33.5 <sup>c,f</sup>	52.0 <sup>d,f</sup>	34.3 <sup>c,f</sup>
Corn	68.6 <sup>d,e</sup>	35.0 <sup>c,e</sup>	73.3 <sup>d,e</sup>	55.2 <sup>c,e</sup>	75.8 <sup>d,e</sup>	59.0 <sup>c,e</sup>	77.2 <sup>d,e</sup>	63.0 <sup>c,e</sup>

<sup>a</sup> IVRDMD was the percent in vitro rumen dry matter digestibility.

<sup>b</sup> IVCDMD was the percent in vitro cecal dry matter digestibility.

<sup>c,d</sup> Means in the same row with different superscripts for each period differ ( $P < .05$ ).

<sup>e,f,g</sup> Means in the same column with different superscripts differ ( $P < .05$ ).

Table 6. *In vitro* rumen versus cecal organic matter digestibility of ryegrass straw, alfalfa meal and corn incubated for different periods (24, 48, 72 or 96 h).

Substrate	Period of Incubation							
	24 h		48 h		72 h		96 h	
	IVROMD <sup>a</sup>	IVCOMD <sup>b</sup>	IVROMD <sup>a</sup>	IVCOMD <sup>b</sup>	IVROMD <sup>a</sup>	IVCOMD <sup>b</sup>	IVROMD <sup>a</sup>	IVCOMD <sup>b</sup>
Annual ryegrass straw	34.0 <sup>d,c</sup>	9.7 <sup>c,o</sup>	44.7 <sup>d,o</sup>	16.7 <sup>c,o</sup>	50.2 <sup>d,o</sup>	32.0 <sup>c,e</sup>	52.3 <sup>d,e</sup>	33.7 <sup>c,e</sup>
Alfalfa meal	46.3 <sup>d,f</sup>	24.3 <sup>c,f</sup>	55.7 <sup>d,f</sup>	31.0 <sup>c,f</sup>	57.5 <sup>d,f</sup>	40.0 <sup>c,f</sup>	56.0 <sup>d,f</sup>	40.6 <sup>c,f</sup>
Corn	72.6 <sup>d,g</sup>	37.8 <sup>c,g</sup>	74.7 <sup>d,g</sup>	57.3 <sup>c,g</sup>	78.6 <sup>d,g</sup>	61.1 <sup>c,g</sup>	78.6 <sup>d,g</sup>	65.5 <sup>c,g</sup>

<sup>a</sup> IVROMD was the percent *in vitro* rumen organic matter digestibility.

<sup>b</sup> IVCOMD was the percent *in vitro* cecal organic matter digestibility.

<sup>c,d</sup> Means in the same row with different superscripts for each period differ (P < .05).

<sup>e,f,g</sup> Means in the same column with different superscripts 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 incubated for different periods (24, 48, 72 or 96 h).

Substrate	Period of Incubation			
	24 h	48 h	72 h	96 h
Annual ryegrass straw	21.5	31.4	58.1	58.6
Alfalfa meal	46.8	50.1	62.7	66.0
Corn	51.0	75.3	77.8	81.6

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 incubated for different periods (24, 48, 72 or 96 h).

Substrate	Period of Incubation			
	24 h	48 h	72 h	96 h
Annual ryegrass straw	28.5	37.4	63.7	64.4
Alfalfa meal	52.5	55.7	69.6	72.5
Corn	52.1	76.7	77.7	83.3

### Conclusion

In vitro digestibility of the various substrates was higher than their in vitro cecal digestibility. The optimum period of in vitro rumen fermentation was 48 h while that of in vitro cecal fermentation was 72 h. There is a need to investigate the effects of age of rabbits on the digestibility potentials of inoculum collected from the animals.

Literature Cited

- Aderibigbe, A.O., P.R. Cheeke and N.M. Patton. 1992. In vitro rumen and rabbit cecum digestibilities: I. Effects of probiotics and incubation time. J. Appl. Rabbit Res. 15:948-958.
- Neter, J. and W. Wasserman. 1977. Applied Linear Statistical Models. 8th ed. Richard D. Irwin, Inc., Homewood, IL.
- Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill, New York.