

## EFFECT OF INOCULATING WEANLING RABBITS WITH BOVINE RUMEN FLUID ON PERFORMANCE AND DIET DIGESTIBILITY

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### Abstract

Forty weanling New Zealand White rabbits (24 days of age) were allotted randomly to four treatments (10 animals per treatment) and orally inoculated on days 26 and 30 of age with 5 ml of four treatment solutions using a rat intubation needle attached to a syringe. The treatment solutions were (a) nutrient-buffer solution (control), (b) autoclaved rumen liquor, (c) rumen liquor without protozoa and (d) rumen liquor containing bacteria and protozoa. The animals were fed a standard Oregon State University doe herd diet during a 28 day digestion and performance trial. Seven of the ten animals inoculated with rumen fluid without protozoa died within 24 h after inoculation, indicating that the solution was highly toxic to weanling rabbits. Oral administration of fresh rumen microflora to the rabbits enhanced acid detergent fiber (ADF) and cell contents (CC) digestibilities, and also resulted in a positive effect on the final live weight of the animals. Autoclaving destroyed the microbial activity of the rumen fluid and the effect of inoculating it to rabbits on nutrient digestibility was similar to that observed for the nutrient-buffer solution. Mortality (%) was higher for animals inoculated with fresh rumen microflora than those inoculated with either nutrient-buffer solution or autoclaved rumen liquor with no significant difference between the latter treatments.

### Introduction

Previous work by us (Aderibigbe *et al.*, 1992a,b,c,d) has shown that *in vitro* cecal digestibility of fibrous feeds is lower in rabbits than *in vitro* rumen digestibility. This presumably reflects differences in microflora, because increasing the length of incubation period did not narrow the difference. This suggests that the cecal microflora of rabbits is not highly cellulolytic, a suggestion supported by the commonly observed low fiber digestibility in rabbits (Cheeke, 1987). The objective of this research was to determine if oral administration of rumen microflora to young rabbits during the "window of enteric vulnerability" (Cheeke, 1988) would increase *in vivo* digestion of fibrous diets. If this positive effect could be achieved, perhaps a commercial preparation of "rabbit probiotic" could be developed.

### Materials and Methods

A nutrient-buffer solution (one liter) was prepared as described by Goering and Van Soest (1970) and kept in a covered glass container in an incubator at 39° C. Rumen fluids were obtained from two rumen-fistulated crossbred cows maintained on grass pasture and a high energy concentrate supplement for two weeks prior to collection. Rumen fluids were collected 2 h after the morning feeding and filtered through two layers of cheese cloth into a pre-warmed thermos bottle (39° C). The fluid was divided into three portions. The first portion was placed in a separatory funnel at 39° C for 2 h for total separation into two parts (bacteria and protozoa). The bacteria part was kept at 39° C while the protozoa part was discarded. The second portion was autoclaved for 16 h and kept at 39° C. The third portion which contained bacteria and protozoa was also kept at 39° C. The solutions were used to orally inoculate 40 weanling New Zealand White rabbits (26 days old, average weight 502 g) allotted at random to 4 treatments with 10 rabbits per treatment in a 28-day digestion and performance trial.

The various treatments were (a) control (nutrient-buffer solution), (b) autoclaved rumen liquor, (c) rumen liquor without protozoa and (d) rumen liquor containing bacteria and protozoa. Five ml of inoculum were given to each rabbit during two consecutive days using a rat intubation needle attached to a syringe. The rabbits were weaned at 24 days of age and inoculated at 26 and 30 days of age, respectively, so that no milk remained in the gut when the inoculum was given, because milk contains an antibiotic factor (Cheeke, 1987). The animals were fed a standard Oregon State University doe herd diet (OSU 61). The ingredient and chemical compositions of the experimental diet are shown in Tables 1 and 2, respectively. Seven of the ten animals inoculated with rumen fluid without protozoa died within 24 h after inoculation. Hence, this treatment was observed to be toxic to the animals and was discontinued.

Table 1. Ingredient composition (% DM basis) of the standard Oregon State University doe herd diet (OSU 61<sup>a</sup>) used as the experimental diet.

Ingredient	% Composition (Dry Matter Basis)
Alfalfa meal	60.3
Wheat mill run	31.2
Cane molasses	3.0
Meat meal	1.4
Trace minerals	0.5

<sup>a</sup> OSU 61 also contained 17.8 kg bentonite (pellet binder), 0.9 kg CuSO<sub>4</sub>, 10 million IU vitamin A and 20,000 IU vitamin E per 908 kg.

Table 2. Chemical composition of the standard Oregon State University doe herd diet (OSU 61) used as the experimental diet.

Component	Percent
Dry matter (DM, %)	92.7
Organic matter (OM, %)	79.6
Crude protein (CP, % DM)	15.8
Acid detergent fiber (ADF, % DM)	26.0
Cell contents (CC, % DM)	54.1
Ash (% DM)	13.1

The experimental animals were kept in individual cages equipped with automatic waterers. Live weight of each animal was recorded on days 1, 21, and 28, respectively. Fecal collection screens were attached to the bottom of each cage during the fourth week and total daily feces voided by each animal during this period were kept in labeled plastic bags at 5° C. Cumulative fecal samples of each experimental animal were dried in an oven at 60° C for 48 h. The experimental feed and feces were ground in a Wiley mill (20-mesh screen) and analyzed for dry matter (DM), organic matter (OM), crude protein (CP) and ash by the AOAC (1975) procedures. Acid detergent fiber (ADF) was determined by the method of Van Soest and Marcus (1964). Digestion coefficients for components of each diet were determined by methods described by Schneider and Flatt (1975). Data for the digestion and performance trial were analyzed using the general linear models procedure as described by Neter and Wasserman (1977). Means were compared using Tukey's studentized range test as outlined by Steel and Torrie (1980).

### Results and Discussion

The results showed that rumen fluid containing no protozoa was highly toxic to weanling rabbits at the applied level of inoculation with 7 out of 10 animals receiving this treatment dying within 24 hr after inoculation, with signs of severe diarrhea. We have no explanation for the apparent beneficial effect of the protozoa, or rather, the severe detrimental effect in their absence. Thus this treatment was discontinued.

The % digestibility of diet components by the experimental animals is shown in Table 3. Digestibilities of DM, OM, CP and ash among the animals on the various treatments were not different ( $P > .05$ ). Digestibilities of ADF and cell contents (CC) were higher ( $P < .05$ ) for animals inoculated with fresh rumen liquor containing bacteria and protozoa than for those inoculated with either nutrient-buffer solution or autoclaved rumen liquor with no difference ( $P < .05$ ) between the latter treatments. Thus, oral administration of

Table 3. Digestibility (%) of individual components of the diet by the experimental animals on the different treatments.

Component	Treatment		
	Nutrient-buffer Solution	Autoclaved Rumen Liquor	Fresh Rumen Liquor With Bacteria and Protozoa
Dry matter	55.0	54.1	55.4
Organic matter	51.7	50.6	52.0
Crude protein	72.7	72.5	72.9
Acid detergent fiber	19.7 <sup>a</sup>	19.1 <sup>a</sup>	24.7 <sup>b</sup>
Cell content	77.1 <sup>a</sup>	77.0 <sup>a</sup>	80.4 <sup>b</sup>
Ash	52.3	50.1	51.6

<sup>a,b</sup> Means in the same row with different superscripts differ ( $P < .05$ ).

rumen microflora to young weanling rabbits enhanced ADF and CC digestibilities although the increase (ADF digestibility increased from about 19% to about 24%, while CC digestibility increased from about 71% to about 80%) was moderate. Autoclaving destroyed the microbial activity of the rumen fluid and the effect of inoculating it to rabbits on nutrient digestibility was similar to that observed for the nutrient-buffer solution.

Table 4 shows the results of some selected data on performance characteristics of the experimental animals. The average daily gain, average daily feed intake and average feed conversion of the animals on the various experimental treatments were not different ( $P > .05$ ). Average final live weight (AFLW) was higher ( $P < .05$ ) for animals inoculated with autoclaved rumen liquor than for the other animals. AFLW was also higher for animals inoculated with fresh rumen liquor containing bacteria and protozoa than for those inoculated with nutrient-buffer solution. These indicated that oral inoculation of weanling rabbits with rumen liquor resulted in a positive effect on the final live weight of the animals. Mortality (%) was higher for animals inoculated with fresh rumen liquor containing bacteria and protozoa than for those inoculated with either nutrient-buffer solution or autoclaved rumen liquor with no difference ( $P > .05$ ) between the latter

Table 4. Selected data on performance characteristics of the experimental animals on the different treatments.

Item <sup>a</sup>	Treatment		
	Nutrient- buffer Solution	Autoclaved Rumen Liquor	Fresh Rumen Liquor With Bacteria and Protozoa
Avg initial live wt, g	439.5	578.9	486.1
Avg final live wt, g	1482.3 <sup>a</sup>	1710.8 <sup>c</sup>	1627.7 <sup>b</sup>
Avg live daily gain, g	37.2	40.4	40.8
Avg daily feed intake, g	116.0	129.4	127.8
Avg feed conversion, g feed/g gain (DM basis)	3.1	3.2	3.1
Percent mortality	10.0	10.0	10.0

<sup>a</sup> Values were obtained from surviving animals only.

treatments. Thus, inoculating weanling rabbits with fresh rumen liquor resulted in increased mortality of the animals.

### Conclusions

Oral administration of rumen liquor containing bacteria and protozoa to young weanling rabbits during the "window of enteric vulnerability" increased *in vivo* digestion of ADF and CC, although the increase was moderate. Rumen liquor containing no protozoa was highly toxic to weanling rabbits. Autoclaving destroyed the microbial activity of rumen liquor and the effect of inoculating it to rabbits on nutrient digestibility was similar to that observed for inoculating a nutrient-buffer solution.

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