

## EFFECT OF FRUCTOOLIGOSACCHARIDES AND YEAST CULTURE ON GROWTH PERFORMANCE OF RABBITS

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### Abstract

Several experiments were performed to assess the influence of a fructooligosaccharide (FOS) preparation (50% solids) alone and in combination with the probiotic Lacto-Sacc (LS) on the growth performance of weanling rabbits. A dose response study of group caged animals given up to 25 g/l FOS in the drinking water appeared to show a significant negative correlation between FOS level and feed conversion (FER) but no effect on average daily gain (ADG). Further work failed to reproduce this effect when FOS was offered at 0, 10, 20 or 30 g/l or with the addition of 0, 1.25 or 2.5 g/l LS. Treatments of (1) 5 g/l FOS, (2) 1.25 g/l LS and (3) FOS combined with LS treatments compared to (4) tapwater controls failed to find significant differences in ADG or FER among treatments. FOS and LS were incorporated into high (54%) and low (10%) alfalfa diets at (1) 0%, (2) 0.75% FOS, (3) 0.2% LS and (4) FOS and LS. FER was significantly less in the low alfalfa diet but neither FOS nor LS had any significant treatment effect on ADG, FER, dry matter, crude protein or ether extract digestibilities or volatile fatty acid concentrations of the cecal contents. The FER correlated strongly with the initial bodyweight of the rabbits. Mortality was low among all treatments.

### Introduction

The economic competitiveness of rabbit production depends in part on the utilization of inexpensive feeds and on animals surviving to market weight. Rabbits are inefficient in digestion of fiber and are susceptible to enteritis when fed concentrates (Cheeke 1987). Since corn and soybean based feeds are less expensive in the U.S. than diets based on alfalfa meal, it would be advantageous for rabbit growers to utilize these feed sources. One strategy to achieve these goals is to formulate diets giving selective advantage to beneficial gut flora.

We undertook to investigate the growth response of rabbits given fructooligosaccharides (FOS), a nonstarch polysaccharide not hydrolyzable by *Clostridia spp.* and *E. coli*, in combination with Lacto-Sacc (LS), a commercial yeast culture probiotic. It was hypothesized that FOS in the presence of the LS preparation could influence the growth performance of rabbits through favorable effects on gut microflora. The objectives of these experiments were to determine the average daily gain, feed conversion and mortality in rabbits given these feed additives in combination with high and low roughage diets.

### Material and Methods

The present work was conducted at the OSU Rabbit Research Center with weanling New Zealand White rabbits of both sexes. All animals were kept in hanging wire cages in naturally ventilated

buildings. Animals selected for the studies were ear tagged, weighed and grouped for balanced initial weight. Feed was offered ad libitum in "J" type galvanized metal feeders. Statistical significance was accepted up to the 5% level ( $p \leq 0.05$ ). Fructooligosaccharides (FOS) were a 50% solids solution provided as a gift by ZeaGen Inc., Bloomfield CO (formerly Coors Biotech Inc.). Calculations involving FOS were done on an as is basis. The probiotic Lacto-Sacc (LS) was a gift of Alltech Inc. Nicholasville, KY 40356 containing "dried *Streptococcus faecium* fermentation product, dried *Lactobacillus acidophilus* fermentation product, yeast culture (live *Saccharomyces cerevisiae* grown on a media of ground yellow corn, diastatic malt and cane molasses), dried *Aspergillus niger* fermentation extract, beta glucan (encapsulating agent)".

### Experiment 1.

This experiment was designed to assay the effects of increasing levels of FOS in the drinking water on the growth response of rabbits. Two ranges of FOS concentration were selected, the lower range including 0, 5, 7.5, 10 and 12.5 g FOS/L, the upper range including 0, 10, 15, 20 and 25 g FOS/L. The FOS solutions were offered in crocks and were checked twice daily for fluid level and contamination. The alfalfa based standard OSU fryer diet was offered ad libitum in "J" type galvanized metal feeders. The alfalfa based standard OSU fryer diet was offered ad libitum. The diet composition and proximate analysis are shown in Table 1 (control diet). Three replicates of each range were conducted totalling 120 animals. Each replicate lasted 35 or 36 days. Daily feed and water utilization per pen was recorded. Bodyweights of all animals were taken several times in the course of each replicate.

All responses to levels of FOS were combined into a single regression analysis. The dose response of ADG and FER to FOS level in the drinking water were tested by multiple linear regression analysis with initial bodyweight chosen as the second independent regressor variable. Similarity of initial bodyweights were tested by analysis of variance. Hypothesis testing (F test) was conducted as outlined in Snedecor and Cochran (1989).

Table 1. Composition of Diets (%)

Ingredients	Control	Alfalfa	
		High	Low
Alfalfa meal	54	54	10
Ground corn	--	--	35
Soybean meal	21	12	12
Wheat mill run	21	28	36.50
Molasses	2	3	3
Dicalcium Phosphate	0.25	0.25	0.25
Trace mineral salt	0.50	0.50	0.50
Vitamin premix	--	0.25	0.25
Vegetable oil	1.25	2	2
Limestone	--	--	0.50
Analyzed composition:			
Dry matter	87.6	90.6	89.9
Crude protein	25.3	17.9	16.2
Ether extract	--	4.2	4.9
Ash	--	9.5	9.6
ADF	20.1	23.1	9.9

### Experiment 2.

This experiment was a factorial design utilizing 10 animals per treatment. The objective was to assay the growth effects of combinations of FOS and LS. The treatments included two levels of FOS (0 and 5 g/l) and two levels of LS (0 and 1.25 g/l) supplemented tapwater. The drinking water treatments were made up twice daily and offered ad libitum in watering bottles. Feed was withheld from all animals for 12 h following assignment to individual cages. The control diet of experiment 1 was offered ad libitum. The FER and ADG response means were tested for significance of difference by covariance analysis using a microcomputer statistical routine. Interpretations were made in accordance with Snedecor and Cochran (1989).

### Experiment 3.

This dose response experiment was designed to extend the results of experiments 1 and 2. Combinations of FOS at 0, 10, 20 and 30 g/l, and LS at 0, 1.25 and 2.5 g/l were offered to the animals in water bottles. The water bottles were replenished twice per day. Four individually caged animals were selected per treatment totalling 32 rabbits. The control diet of experiment 1 was offered ad libitum. Statistical analysis was by multiple linear regression with initial weight, FOS and LS levels as independent variables. Hypothesis testing was conducted as outlined in Snedecor and Cochran (1989).

### Experiment 4.

The objective of this experiment was to evaluate responses to FOS and LS when included in the diet. Two basal diets were formulated with either high or low alfalfa content. Feed supplements were incorporated at 0 or 0.75% for FOS and 0 or 0.2% for LS, giving eight dietary treatments. Water was provided through an automatic watering device. Five animals were selected per treatment per replicate, with three replicates were performed giving a total of 120 rabbits. The digestibility of the diets was determined during the last week of the third replicate. At the conclusion of the digestibility collections three animals from each treatment were selected for cecal volatile fatty acid (VFA) analysis. The diet compositions are shown in Table 1. Statistical analysis of initial bodyweight, ADG and FER was by multiple ANOVA using the replicate growth observations as the dependent variables. One way ANOVA was performed on the digestibility and VFA results. The significance of differences among means were made by t-tests based on the pooled estimate of experimental error.

## Results and Discussion

### Experiment 1.

Table 2 shows the observed ADG and FER for all replicates. Because of the order of replicates and the overall availability of animals during the experimental period, the animals on the lower treatment levels tended to have higher initial mean weights although these weights were not significantly different by 1 way ANOVA ( $p=0.81$ ). Initial bodyweight did not correlate with ADG and provided little improvement of regression fit over FOS level alone. ADG also correlated poorly with FOS level ( $r^2=0.091$ ) and the regression did not have a significant slope.

FER correlated most strongly with initial bodyweight (positive correlation) although introduction of FOS level into the regression model significantly improved the fit (negative correlation). Over the range of FOS levels used, it appeared that feed conversion could be significantly improved with the introduction of FOS in the drinking water.

Table 2. Average daily gains (ADG, g/d) and feed conversion (FER, g feed/g wt. gain) in rabbits offered increasing levels of fructooligosaccharides in the drinking water (experiment 1).

FOS g/l	Replicates	Animals	Initial Weight	ADG	FER
0	6	22	1004 ± 153	38.2 ± 3.7	4.1 ± 0.4
5	3	11	1118 ± 98	41.8 ± 3.5	4.0 ± 0.2
7.5	3	12	1057 ± 85	38.2 ± 3.1	4.1 ± 0.2
10	6	23	973 ± 196	41.1 ± 4.8	3.8 ± 0.3
12.5	3	12	1023 ± 135	39.7 ± 3.6	3.9 ± 0.2
15	3	12	990 ± 140	41.3 ± 2.5	3.7 ± 0.4
20	3	12	963 ± 150	42.3 ± 1.7	3.6 ± 0.2
25	3	11	930 ± 98	40.3 ± 2.6	3.6 ± 0.1

### Experiment 2.

Table 3 shows the initial weights, ADG, FER and statistical parameters for the analysis of experiment 2. The initial weights were similar and covariance adjustment had little effect.

The ADG and FER of treatment groups were not significantly different from the tapwater control groups. These results are in agreement with those of experiment 1. However, the level of FOS used in the current study was deliberately set low to achieve intakes comparable to those recommended for poultry (ZeaGen, Inc.). In experiment 1 the performance of rabbits offered FOS at 5 g/l was not appreciably improved above tapwater controls.

Table 3. Weights, average daily gains (ADG, g/d) and feed conversion (FER, g feed/g weight gain) in rabbits offered fructooligosaccharides (FOS) and probiotic (LS) in the drinking water (mean ± SD) (experiment 2).

	GROUP				p	MSE
	Water	FOS	LS	LS+FOS		
n	10	10	10	10		
Initial wt.	907 ± 98	952 ± 73	888 ± 100	949 ± 94	0.33	8468
ADG	38.7 ± 2.7	36.4 ± 3.6	38.7 ± 5.6	37.6 ± 2.0	0.51	14.3
FER	3.74 ± 0.34	3.88 ± 0.26	3.78 ± 0.31	3.85 ± 0.38	0.92	.0747

### Experiment 3.

The results of experiment 3 are summarized in Table 4. A significant negative correlation existed between FOS and ADG due largely to a negative correlation between ADG and FOS in the presence of 2.5 g/l LS. This correlation did not exist at 1.25 g/l LS suggesting a possible deterioration of the drinking water solution at high concentrations of yeast culture and substrate. The fit of the regression model was not significantly improved with the introduction of LS or initial bodyweight which indicated that LS and IBW had little overall effect on ADG in these rabbits.

Feed conversion in these rabbits was almost entirely dependent on initial bodyweight. The strongest correlation with FER was with initial bodyweight and neither the introduction of LS nor FOS into the regression model significantly improved the fit. These results agree with experiment 1 where decreased FER correlated with decreased initial bodyweight although the improvement in FER with increasing FOS levels in experiment 1 did not hold in experiment 3. Unlike ADG, FER did not significantly correlate with the level of LS in the drinking water.

Experiment 4.

Table 5 shows the growth responses to the high and low alfalfa diets in combination with FOS and LS. For comparison, 0.75% dietary FOS is approximately equivalent to 5 g FOS/L and 0.2% dietary LS is approximately equal to 1.25 g LS/L. By one way ANOVA the initial bodyweights were not significantly different among treatment groups. Neither the alfalfa content nor the addition of FOS or LS significantly influenced ADG although gains tended to be higher in rabbits fed high alfalfa diets. Hollister et al. (1990) also found no effect on ADG with 0.1% dietary LS; however, significant improvement of FER did occur.

Table 4. Average daily gains (ADG, g/d), feed conversion (FER, g feed/g weight gain) and initial bodyweight (IW, g) of rabbits offered fructooligosaccharides (FOS) and probiotic (LS) in the drinking water (mean ± SD) (experiment 3).

	LS (g/l)					
	0	1.25	2.5	0	1.25	2.5
<u>FOS</u> 0 g/l				<u>FOS</u> 10 g/l		
ADG	36.3 ± 2.7	36.2 ± 3.9	37.9 ± 5.0	--	36.0 ± 3.0	37.3 ± 1.7
FER	3.7 ± 0.3	3.4 ± 0.3	3.4 ± 0.3	--	3.2 ± 0.3	3.2 ± 0.2
IW	711 ± 68	600 ± 177	606 ± 117	--	563 ± 132	544 ± 121
<u>FOS</u> 20 g/l				<u>FOS</u> 30 g/l		
ADG	--	35.1 ± 2.5	33.7 ± 3.1	--	36.3 ± 1.5	30.7 ± 5.3
FER	--	3.3 ± 0.3	3.3 ± 0.2	--	3.4 ± 0.1	3.3 ± 0.2
IW	--	619 ± 101	612 ± 186	--	670 ± 94	622 ± 133

As expected, FER was significantly poorer on the low roughage diets although the FOS and LS treatments did not significantly affect feed conversion. In spite of the similarity of group mean initial weights FER was significantly correlated with initial bodyweight. Mortality levels were low across all treatments and combined with the relatively low sample sizes do not provide a clear picture of the influence of FOS and LS on enteritis. Hollister et al. (1989) noted reduction of mortality in rabbits with 0.1% dietary LS. There was very little enteritis in the present experiments, so effects of FOS and LS on enteric disease could not be evaluated.

As shown in Table 6, treatment means for digestibilities were not significantly affected. As expected (Cheeke 1987), significant differences did appear between diet types, these treatment and diet effects were in agreement with the results of Hollister et al. (1989).

Table 5. Average daily gains (ADG, g/d), feed conversion (FER, g feed/g weight gain) and initial bodyweight (IW, g) of rabbits offered 0.75% fructooligosaccharide (FOS) and 0.2% probiotic (LS) diluted feed (mean  $\pm$  SD) (experiment 4).

	Control	FOS	LS	FOS/LS
<b>High Alfalfa</b>				
n	15	15	15	15
ADG	37.4 $\pm$ 5.4	36.3 $\pm$ 5.0	37.5 $\pm$ 5.2	37.1 $\pm$ 2.8
FER	3.6 $\pm$ 0.4	3.8 $\pm$ 0.5	3.7 $\pm$ 0.4	3.8 $\pm$ 0.4
IW	742 $\pm$ 174	742 $\pm$ 183	807 $\pm$ 183	830 $\pm$ 191
<b>Low Alfalfa</b>				
n	14	15	14	12
ADG	29.6 $\pm$ 6.0	29.3 $\pm$ 7.4	26.7 $\pm$ 6.9	33.4 $\pm$ 5.6
FER	3.2 $\pm$ 0.4	3.2 $\pm$ 0.6	3.5 $\pm$ 0.9	3.1 $\pm$ 0.4
IW	804 $\pm$ 195	745 $\pm$ 179	798 $\pm$ 247	809 $\pm$ 180

As shown in Table 7, total VFA tended to be higher in the cecal contents of rabbits fed the high alfalfa diets, although propionate was significantly higher in the low alfalfa diet. There were no significant differences among treatment means. High starch diets can be expected to increase cecal VFA levels in rabbits but may lead to hypomotility, a point supported by the greater digestibilities seen in the low fiber diet (Cheeke 1987). Only the FOS fed low fiber (low alfalfa) animals had VFA levels approaching those of the high fiber animals, largely attributable to increased acetate and butyrate. In spite of the unexpected distribution of VFA, gut hypomotility may still have prevailed in the low fiber diets resulting in greater VFA absorption. Given the equivalency and adequacy of the dietary protein, it is unlikely that it played a major role in the differences in VFA production, ADG and FER.

Table 6. Digestibility of diets containing fructooligosaccharide (FOS, 0.75%) and probiotic (LS, 0.2%) in rabbits (experiment 4)<sup>1</sup>.

	Control	FOS	LS	FOS/LS
<b>High Alfalfa</b>				
n	5	5	5	5
Dry matter	49.7 $\pm$ 6.7	53.2 $\pm$ 1.9	53.8 $\pm$ 1.4	48.6 $\pm$ 7.0
Crude Protein	64.3 $\pm$ 4.6	68.1 $\pm$ 5.9	67.6 $\pm$ 3.1	62.3 $\pm$ 3.8
Ether extract	66.9 $\pm$ 8.2	64.9 $\pm$ 4.2	66.6 $\pm$ 2.5	64.5 $\pm$ 4.6
<b>Low Alfalfa</b>				
n	5	5	5	5
Dry matter	72.3 $\pm$ 3.6	73.0 $\pm$ 1.9	74.5 $\pm$ 1.9	74.8 $\pm$ 4.9
Crude Protein	714. $\pm$ 3.3	72.1 $\pm$ 2.6	74.1 $\pm$ 3.6	72.3 $\pm$ 6.5
Ether extract	83.1 $\pm$ 1.6	86.6 $\pm$ 1.2	87.7 $\pm$ 1.2	84.2 $\pm$ 6.4

<sup>1</sup> Mean  $\pm$  SD. No treatments within diets differed significantly although mean digestibility was significantly different between diets in all analyses.

Fuller (1986) noted that beneficial effects of probiotics depend on growth of the probiotic organism in the host animal. Since we did not survey the gut flora we do not know if the probiotic organisms became established in the gut. Although the VFA profile was influenced by the diets the FOS and LS treatments produced no significant treatment differences. It was possible that the benefits of FOS and LS were not apparent at the scale of the present experiments.

Table 7. Volatile fatty acid content (mM/kg) of the cecal contents of rabbits fed 0.75% fructooligosaccharide (FOS) and 0.2% probiotic (LS) diluted feed (mean  $\pm$  SD) (experiment 5).

	Control	FOS	LS	FOS/LS	Mean
<b>High Alfalfa</b>					
Acetate	69.5 $\pm$ 6.5	54.0 $\pm$ 14.6	67.3 $\pm$ 4.5	64.0 $\pm$ 17.9	63.7
Propionate	4.6 $\pm$ 1.2	5.9 $\pm$ 2.5	4.0 $\pm$ 0.1	4.4 $\pm$ 2.6	4.7
Butyrate	15.1 $\pm$ 1.6	14.1 $\pm$ 8.3	14.7 $\pm$ 0.7	14.2 $\pm$ 9.3	14.5
Valerate	0.5 $\pm$ 0.1	0.8 $\pm$ 0.3	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6
Total VFA	89.7	74.8	86.6	83.1	83.6
<b>Low Alfalfa</b>					
n	3	3	3	3	
Acetate	56.5 $\pm$ 13.7	61.0 $\pm$ 20.8	51.2 $\pm$ 10.1	49.9 $\pm$ 2.1	54.5
Propionate	3.2 $\pm$ 0.4	3.6 $\pm$ 0.7	2.9 $\pm$ 0.2	2.9 $\pm$ 0.3	3.2
Butyrate	10.7 $\pm$ 5.9	19.5 $\pm$ 5.6	11.1 $\pm$ 6.1	13.7 $\pm$ 0.5	13.8
Valerate	0.5 $\pm$ 0.1	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	0.4 $\pm$ 0.3	0.6
Total VFA	70.9	84.9	65.8	66.9	72.1

### Summary

The current results provided some evidence that FOS influenced the ADG and FER of weanling rabbits. The interpretation of FER was consistently difficult due to the sensitivity of this index to the initial weight of the animals. Nevertheless, a dose response relationship was established in group caged animals between FER and FOS level up to 25 g/l in the drinking water. We were not able to reproduce this effect among individually caged animals offered FOS up to 30 g/l (experiment 3) nor was FER significantly different between treatment animals offered 5 g/l FOS and tapwater controls (experiment 2). When FOS was incorporated in feed at 0.75% no significant differences were detected between treatment and control ADG or FER.

ADG and FER were not significantly effected by LS in any of the experiments. The effects of high versus low fiber diets were immediately apparent in ADG and FER, and in an unexpected manner in VFA production. Larger samples sizes were probably required to detect any effects of FOS and LS on ADG and FER. Stress factors such as heat and transportation which may be alleviated by probiotics (Sissons 1989) could be incorporated into future work.

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