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## **An Evaluation of the Ruminal Establishment of *Propionibacterium* species in Dairy Cattle Using Three Different Treatments**

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### **INTRODUCTION**

Select *Propionibacterium* strains have been shown to influence ruminal parameters when fed to beef cattle. Cannulated beef cattle treated with strain P-63 for 14 days had higher ruminal pH, lower lactic acid, and more consistent lactic acid producing microbial populations following a diet challenge with a highly fermentable carbohydrate ration. Feeding strain P-5 for 30 days has been shown to reduce ruminal nitrite levels and prevent cattle from dying when fed rations with nitrate levels up to 20,000 ppm. Additionally, cattle have been shown to have improved overall feed efficiency compared to cattle with no treatment. In previous trials, ruminal establishment of the bacteria at specific levels has been found crucial to achieving desired results.

Improved ruminal parameters are expected in high producing dairy cattle due to the high energy components used in the ration formulation of these herds. This examination was conducted to determine the establishment of *Propionibacterium* strain P-63 when administered to dairy cows in three different modes.

### **MATERIALS and METHODS**

#### **Cows**

A total of 12 multiparous Holstein cows were randomly assigned to one of three treatment groups: 1) high level of *Propionibacterium* strain P-63 daily for 7 days, 2) low level of P-63 daily for 7 days, or 3) low level of P-63 in a gelatin capsule on days 5, 6 and 7 only. In previous research,  $10^4$  to  $10^6$  CFU/ml of rumen fluid has been administered to beef cattle figuring 30 liter rumen capacity. The low and high levels used in this study represent the limits of this range figuring a 60 liter rumen for an adult dairy cow.

#### **Ruminal fluid collection**

Ruminal fluid was collected by oral esophageal-rumen recovery using a soft, flexible rubber hose and a vacuum pump.

Esophageal-rumen tubes were soaked in concentrated chlorine bleach and rinsed with water between animals to prevent cross contamination of microbial populations. Flasks containing the acquired ruminal fluid were stored on ice packs and immediately transported to the laboratory for selective bacterial analysis.

Cows were pre-screened for and determined to be free of native *Propionibacterium* populations. To determine the populations of established *Propionibacterium* strain P-63 following inoculation, ruminal fluid was collected from each cow 7 days after the completion of the treatment period (14 days following the start of the examination).

### **RESULTS and DISCUSSION**

The ruminal populations of *Propionibacterium* strain P-63 in cows 7 days after the last treatment are shown in Table 1. This data indicates that *Propionibacterium* was detected in 100% of the cows treated with all three modes of application and doses.

Cow #78 (group 2) had the highest established population of all animals tested, however, cow #6 (also in group 2) had the lowest population. No relationship between populations at the end of the trial and records of the actual amount of inoculum consumed by individual cows could be established. For example, cow # 80 refused or only partially consumed the inoculum on the final 5 days of the 7 day treatment period, yet this cow had the highest *Propionibacterium* population in group 1 and second highest overall. Cow #6 consumed the entire inoculum on all but the last of the 7 days of treatment, yet had the lowest *Propionibacterium* population of all cows.

Previous studies have established the effective *Propionibacterium* populations in the rumen at between  $10^4$  and  $10^6$  CFU/ml of rumen fluid. This level was achieved in only 50% of the cows in this study. This would suggest longer term feeding to be more effective at

establishing *Propionibacterium* populations in the rumen of dairy cows. Alternatively, the *Propionibacterium* may have been established and populations subsequently decreased during the 7 day period between treatment and ruminal sampling. This may indicate that continuous feeding is more advantageous than short term or pulse feeding of P-63 for maintaining high enough ruminal populations of *Propionibacterium*. Additional study in this area is necessary.

**Table 1.** Ruminal populations of *Propionibacterium* following treatment with strain P-63.

Cow #	Treatment Group	Propionibacterium (CFU per ml)
4	1	5,000
26	1	40,000
80	1	50,000
2	1	4,500
78	2	100,000
10	2	4,000
6	2	500
53	2	3,000
74	3	10,000
54	3	8,000
28	3	10,000
48	3	20,000

Treatment population means are presented in Table 2. Cows in treatment groups 1 and 2 had similar mean populations. Treatment group 3 that were fed the capsule for only 3 days had a 50% lower mean population of ruminal *Propionibacterium* compared to the other two groups. This data indicates the length of time of overall treatment period may be a more critical factor in determining the ruminal establishment of *Propionibacterium* strains compared to the activity of the daily inoculum, with longer periods more likely to result in establishment.

Ruminal *Propionibacterium* populations in group 3 were more consistent than those in either group 1 or 2. This may be due to known

delivery of the P-63 inoculum through the gelatin capsules being forcibly administered to the animals with a balling gun. Alternatively, although most research indicates that gelatin capsules dissolve within minutes of entering the rumen, the capsules administered in this study may have floated on top of the rumen mat and dissolved over a long time period of hours or days. The ration fed these cows consisted of large amounts of long stem hay and balage (70% or more of the ration). This creates a rumen with a high percentage of large particle lengths, which would create a more substantial rumen mat than with typical rations. A substantial rumen mat could possibly suspend a gelatin capsule above the fluid phase necessary to dissolve the gelatin for extended periods of time.

**Table 2.** Mean ruminal *Propionibacterium* populations for each treatment group.

Treatment Group	Propionibacterium (CFU per ml)
1	25,000
2	27,000
3	12,000

## CONCLUSIONS

*Propionibacterium* strain P-63 was established in 100% of dairy cows with all three application modes used in this study including via gelatin capsule for as little as 3 days. Establishment appears more related to duration of treatment than activity of inoculum or total numbers of bacteria administered. Data suggest that continuous feeding of *Propionibacterium* strain P-63 may be advantageous for maintaining ruminal populations at levels indicated by previous research to provide effective results.

## REFERENCES

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