RUMEN MANIPULATION: COMPARISON OF MICROBIAL DYNAMICS AND FORAGE INTAKE OF DEFAUNATED VERSUS NORMALLY FAUNATED GOATS (*Capra hircus* L.) WITH OR WITHOUT PROBIOTIC SUPPLEMENTATION

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ABSTRACT

Feed efficiency can be improved by changing diet composition though probiotic supplementation and changing key microbial groups through defaunation. This study assessed probiotic supplementation as a way of optimizing rumen defaunation in goats fed low-quality forage. The following treatments were compared in this study: T_1 (intact without probiotic supplementation), T_2 (intact with probiotic supplementation), T_3 (defaunated without probiotic supplementation), and T_4 (defaunated with probiotic supplementation). Six out of the twelve animals were defaunated using ipil-ipil (*Leucaena leucocephala*) fed for 9 days; the other 6 were fed Napier grass (*Pennisetum purpureum*) alone. After defaunation, three of the defaunated goats were given probiotic supplement while the other three were not. The same was done to faunated goats. Probiotic supplementation to defaunated goats was successful in increasing protozoal counts ($P<0.01$) and bacterial counts ($P<0.01$) as well as stabilizing the pH ($P<0.01$) of these animals fed low-quality forage. Probiotic supplementation is recommended as a technique to combat the negative effects of defaunation on microbial counts and pH in animals fed low-quality forage.

Keywords: defaunation, ipil-ipil, microbial count, probiotics

INTRODUCTION

Ruminants have evolved a special system of digestion that involves microbial fermentation of food before exposure to the animals' digestive enzymes (McDonald *et al.*, 2010). As a result, biomass that otherwise could not have been digested by the host becomes degraded and is converted to digestible microbial matter, volatile fatty acids (VFA), fermentation gases (CO_2 and CH_4), and heat (Bannink and Tamminga, 2005). The major end-products of fermentation deliver most of the metabolizable energy and metabolizable protein to the host.

The microbial community inhabiting the gastrointestinal tract is characterized by its high population density, wide diversity, and complexity of interactions (Mackie *et al.*, 2000). The rumen, the most extensively studied gut ecosystem, contains predominantly fermentative populations of microorganisms (Theodorou and France, 2005).

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Of the rumen microorganisms, protozoa are generally considered nonessential to the survival and growth of ruminants (Hobson & Stewart, 1997; Ushida *et al.*, 1991), and efforts in rumen manipulation have focused on defaunation or the selective removal of protozoal microfauna from the rumen. Defaunation as rumen manipulation technique can be seen as an optimization process, which can be achieved through minimization and/or maximization of fermentation (Santra and Karim, 2003). For instance, in defaunated animals there is a reduction in ruminal protein degradation (minimization), so more dietary protein (maximization) becomes available for intestinal digestion.

This is especially important in the tropics, where most ruminants are fed low-quality roughages, agricultural crop-residues, and industrial by-products, which basically contain high levels of lignocellulosic materials, low levels of fermentable carbohydrates, and low levels of good-quality protein (Wanapat, 2000). Therefore, efficient but cost-saving farm management practices geared toward improving ruminal fermentation of low-quality roughages can augment the production of smallholder ruminant farmers whose performance is hampered by feed scarcity.

Probiotic preparations have shown promising results in a variety of animal production areas (Whitley *et al.*, 2009). A probiotic is defined as a live microbial food supplement that beneficially affects the host animal by improving the normal rumen fermentation (Broderick *et al.*, 1991) and intestinal microbial balance (McDonald *et al.*, 2010).

While there are several studies on defaunation and on probiotic supplementation in ruminants, none have been on investigating the combined effects of these two rumen manipulation techniques on microbial dynamics and forage intake of ruminants. Feed efficiency can be improved through changes in diet composition brought about by probiotic supplementation and in changes in key microbial groups by defaunation.

This study aimed to assess the viability of the probiotic supplement, compare the effects of probiotic supplementation on rumen microbial dynamics of defaunated versus normally faunated or intact goats, and compare the effects of probiotic supplementation on forage intake of defaunated versus normally faunated goats.

MATERIALS AND METHODS

A day before the start of the study, animals were dewormed. The animals were housed in individual pens to avoid direct contact with pasture, soil, and other animals for possible reinfection after treatment. Pens were cleaned and disinfected prior to use. Likewise, pens were washed and cleaned daily to maintain animal health and well-being. Provisions of Republic Act 8485 or the Animal Welfare Act of 1998 were carefully followed in the care and management of animals in this study.

Twelve female native goats of post-weaning age, 7–12 kg in weight were allocated to 4 treatments arranged in a completely randomized experimental design with three replicates each. The number of animals was computed using the resource method equation based on the law of diminishing return described by Charan and Kantharia (2013).

The following treatments were used in this study:

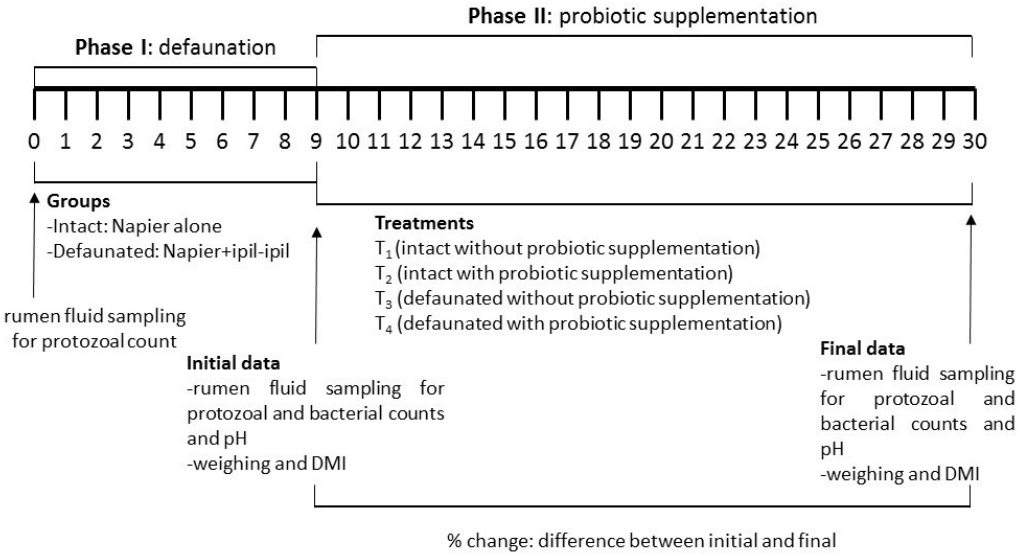
T_1 - intact without probiotic supplementation
T_2 - intact with probiotic supplementation
T_3 - defaunated without probiotic supplementation
T_4 - defaunated with probiotic supplementation

A total of six animals were enrolled in the defaunation protocol using fresh ipil-ipil (*Leucaena leucocephala* Lam. De wit) as a defaunating agent, which is comparable in effects to the chemical agent sodium lauryl sulfate (SLS) but with no toxic effects.

Ratio of Napier grass (*Pennisetum purpureum* Schumach) to ipil-ipil was computed to 3% of body weight expressed on a dry matter (DM) basis such that each forage was given at 1.5% of BW divided into two equal feedings in the morning and afternoon. During each feeding, Napier grass was offered first with ipil-ipil given after. Animals were fed *ad libitum* for 9 days since the rumen is stabilized on the 9th day after defaunation. The other six animals were fed only Napier grass *ad libitum* in the morning and afternoon, and thus remained faunated or intact.

After the defaunation period of 9 days, a total of six animals were supplemented with a commercial probiotic product (RPL-8+AKE) given via oral stomach tubing at a recommended dose of 20 mL per day given in two divided doses. Supplementation was done before each feeding of fresh forage in the morning and afternoon. Animals were given the probiotic product for 3 weeks. As cited by Leek (1993), it takes 2 weeks for the new population of microbial species and numbers to become established when there is a change in diet. The probiotic product was tested for the presence of microorganisms before using. Figure 1 presents a summary of study schedule and pertinent data taken during specific times.

Rumen fluid samples were aspirated via oral stomach tubing connected to a syringe before the start of the study period, after 9 days of defaunation (Phase I), and after 3 weeks of feeding the treatment diets (Phase II). Ten ml of rumen fluid was collected in the morning prior to laboratory analysis. Samples were placed in a screwcap bottle filled up to the brim to prevent oxygen from entering and action of aerobic microorganisms. Samples were brought to the laboratory for protozoal and bacterial counting as well as for pH measurement using a digital pH meter.



Note: Intact goats remained faunated during Phase I.

Figure 1. Schedule of study phases and pertinent data taken.

Bacterial counting was performed through the serial dilution and plate count method (Falkow *et al.*, 2006). Protozoal cell counting, on the other hand, was done using a modified method by Purser and Moir (1959) using a Sedgewick Rafter counting chamber.

Animals were weighed daily. Feed was weighed prior to and after feeding. Dry matter content was analyzed in the laboratory using a convention oven set at 100ºC for 24 hrs (Bestil, 2009) for every type of forage given. Dry matter intake (DMI) and DMI as percent of BW were computed.

Percent change was used for all data collected. This was computed by subtracting the initial (Day 9) from final (Day 30) values, dividing the difference by the initial value (Day 9), and multiplying the quotient by 100. Positive and negative values refer to increases and decreases, respectively. As shown in Figure 1 above, initial counting was done on Day 9 after the defaunation protocol while final counting was done on Day 30 for all data measured.

Statistical Package for the Social Sciences (SPSS) version 23 software was used to analyze the data gathered in this experiment. One-way analysis of variance (ANOVA) was performed and comparison of mean percent change (increases or decreases of Day 30 values from Day 9) of the different treatments for all data collected was then done using Tukey's Honest Significant Difference (HSD) test.

RESULTS AND DISCUSSION

Only yeast and two kinds of bacterial colony were found associated with the probiotic supplement. No mold growth was observed. Colony growth of the isolates and shape of their cells are shown in Figure 2.

Figure 3 shows the increases and/or decreases in mean protozoal counts for each phase of the study. Day 1 shows the protozoal counts of animals during the start of the study. Day 9 values are the post-defaunation initial values, while Day 30 are the final values after the different treatments were given for 3 weeks. Although numbers from Day 1, Day 9, and Day 30 are presented to show increases and decreases during different phases of the study, only the percent change from Day 9 to Day 30 data is meaningful since this

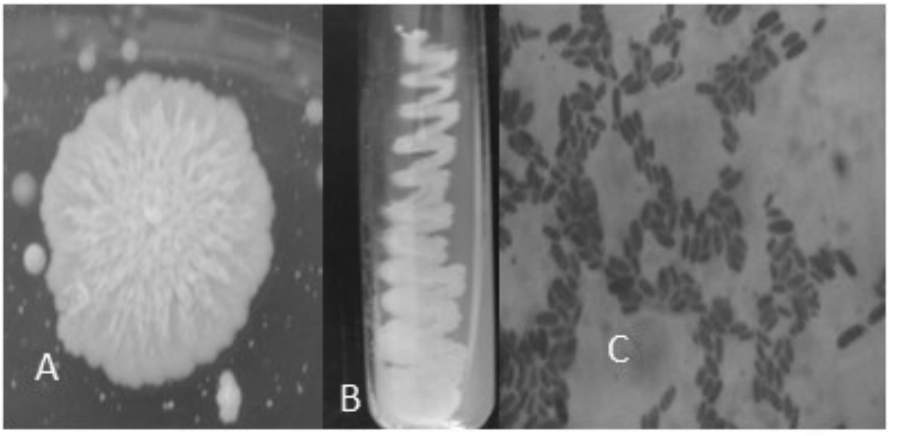


Figure 2. A) Colony growth, B) pure isolate in PDA, and C) cells of yeast isolate.

study investigated the effects of probiotic supplementation on defaunated animals. Table 1 lists these percent changes for the different data gathered.

After analysis, highly significant differences (P=0.001) existed between the mean percent change in protozoal counts of the study treatments. The mean percent change in protozoal counts of defaunated goats supplemented with probiotic (T_4) was highest (Table 1) showing that supplementation of probiotics resulted in higher number of protozoa after defaunation compared to animals that refaunate naturally. The highest increase in protozoal numbers in defaunated animals can be attributed to the yeast contained in the probiotic supplement, which was 8.9×10^6 CFU/ml as determined from the viability testing. Certain strains of active dry yeast are particularly effective in stimulating certain populations of ciliate protozoa, which rapidly engulf starch and, thus, effectively compete with amylolytic lactate-producing bacteria (Owens *et al.*, 1998; Uyeno *et al.*, 2015).

Higher mean percent change in the protozoal count of defaunated animals was expected since goats were only partially defaunated. This means that protozoa were not completely eliminated in the rumen. With doubling time of 5.5 hours as cited by Lynn (2007), protozoa numbers are expected to increase after a certain time.

In terms of percent change in bacterial counts, significant differences (P=0.001) were found between the means of the different treatments. The mean percent change in bacterial count of defaunated goats supplemented with probiotic was the highest. Both mean percent chance of T_4 and T_3 were significantly higher than the other two treatments (Table 1).

Figure 4 depicts the increases and decreases of bacterial counts of the four treatments during different phases of the study. Defaunation with ipil-ipil resulted in a subsequent decrease in bacterial counts after Phase I and before probiotic supplementation

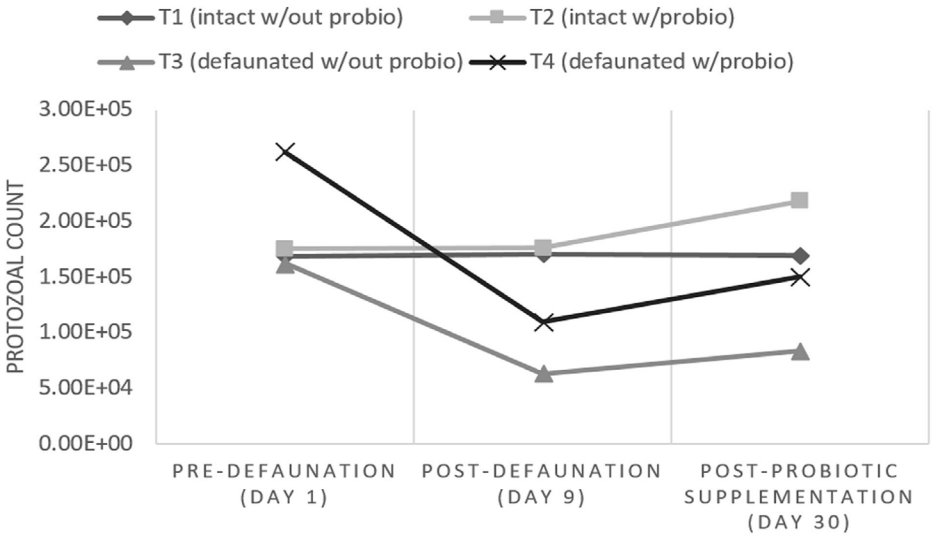


Figure 3. Effects of probiotic supplementation on mean ruminal protozoal counts of faunated versus defaunated goats.

Table 1. Percent change in the mean values for protozoal and bacterial counts and pH.

Treatments	% Change		
	Protozoal Count	Bacterial Count	pH
T_1 (intact w/out probio)	1.48[c]	–10.86[c]	–0.14[b]
T_2 (intact w/probio)	23.75[b]	22.52[b]	0.99[b]
T_3 (defaunated w/out probio)	32.83[b]	38.23[a]	4.95[b]
T_4 (defaunated w/probio)	36.59[a]	44.33[a]	12.00[a]
P - value	0.001**	0.001**	0.004**

Means of the same superscript within a column are not significantly different from each other.
**highly significant

due to its saponin content that affects not only protozoa but bacteria as well. The increased percentage change in the bacterial count of defaunated animals after Phase II as compared with faunated goats was a result of the elimination of a portion of the competing protozoal population to bacterial species in the rumen. Protozoa digest bacteria as their main protein source and as much as 10^2–10^4 bacteria are estimated to be engulfed by a single protozoa per hour as documented *in vitro* (Ushida *et al.*, 1991). Elimination of a certain percentage of protozoal population caused a subsequent increase in bacterial numbers. The higher percent change from initial bacterial counts in T_4 and T_2 as compared to their counterparts that were not supplemented with probiotic can be attributed to the yeast in the supplement. Both the results of protozoal and bacterial counts support the hypothesis that live yeast supplementation accelerates maturation of the rumen microbial ecosystem (Chaucheyras-

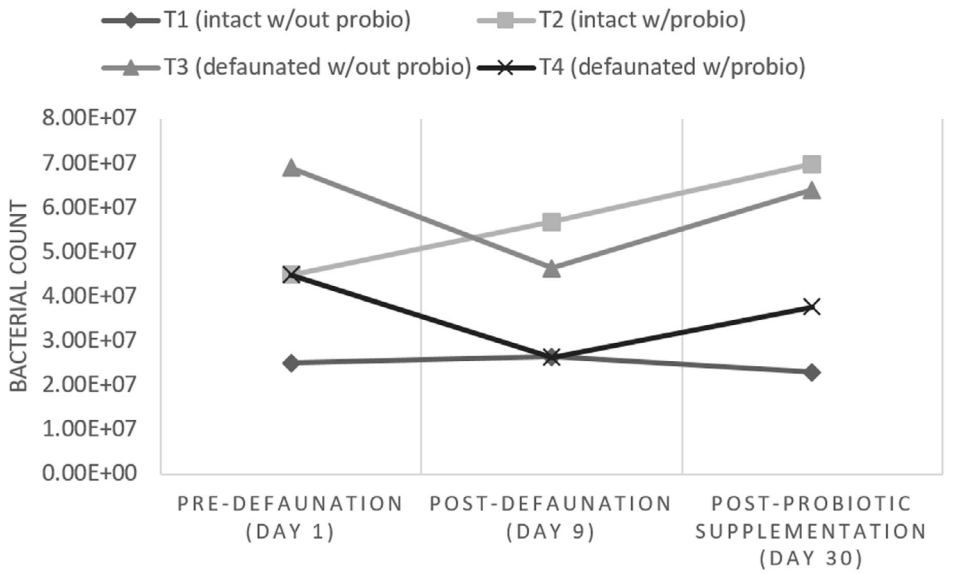


Figure 4. Effects of probiotic supplementation on mean ruminal bacterial counts of faunated versus defaunated goats.

Durand *et al.*, 2012). According to McDonald *et al.* (2010), the precise means by which this effect is achieved have not yet been confirmed, but there are a number of probable mechanisms such as the provision of growth factors as well as the removal of starch and/or sugars as well as hydrogen, all of which lead to increased microbial numbers and activity.

In terms of percent change in pH, Figure 5 shows that pH values taken during different phases of the study. Defaunated animals supplemented with probiotic (T_4) had a significantly higher (P=0.004) percent change in pH compared to other treatments (Table 1). Final mean pH of T_4 was 6.71 and together with the mean pH of T_2 were significantly different from the means of other treatments. These pH values are well within the limit of the optimal ruminal pH of 6.2 or greater of which most rumen microorganisms thrive (Leek, 1993). Even initial pH values after defaunation did not go down below the 5.6 limit suggesting that unlike chemical agents that perturb the rumen ecosystem and animal health to the extreme of being lethal (Frumholtz, 1991), forage with secondary plant metabolites do not cause such adverse effects. Furthermore, stabilization of ruminal pH in the presence of yeast probiotics has been reported by several authors (Bach *et al.*, 2007; Marden *et al.*, 2008). Live yeasts ferment sugars derived from the degradation of starch, thus competing with lactic-acid-producing bacteria, thereby stabilizing rumen pH and reducing the risk of acidosis (McDonald *et al.*, 2010). This impact of yeast probiotics on ruminal lactate concentration has already been confirmed *in vivo* (Chaucheyras-Durand *et al.*, 2012). Initially, the decrease in pH after Phase I defaunation period was due to the elimination of protozoa, which curb undesirably high rates of starch degradation by amylolytic bacteria and prevent lowering of rumen pH (Leek, 1993). The loss of a portion of protozoal population due to the action of saponin caused a subsequent decrease in pH perhaps due to the proliferation of amylolytic bacteria that produce mostly lactic acid, a stronger organic

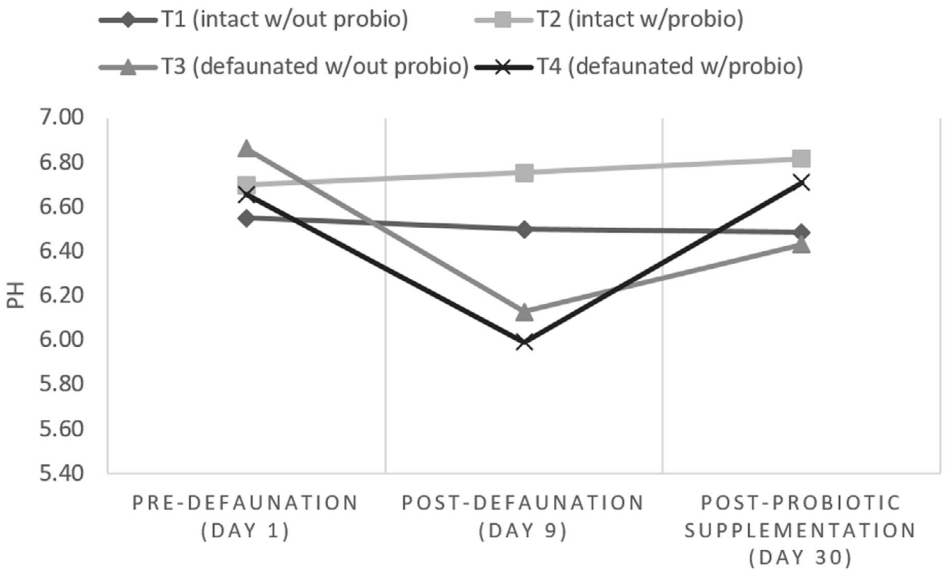


Figure 5. Effects of probiotic supplementation on mean ruminal pH of faunated versus defaunated goats.

acid. Higher percent change in the mean pH of defaunated goats as compared to their faunated counterparts after the 21-day Phase II study period could be due to the subsequent increase in protozoal numbers, which prevented an increase in amylolytic bacteria and increased rumen pH to the ideal.

Figure 6 shows the dry matter intake during the start and end of Phase II probiotic supplementation period of this study as well as the percent change between these two periods. As shown, there was no significant difference in the final DMI (P=0.644) between treatments. These results are similar to other studies conducted to show the effects of probiotics on DMI. According to Bach *et al.* (2007), probiotic supplementation seems to have an effect on intake pattern rather than on intake *per se*. Similar to the results of the dry matter intake of goats, DMI as percent of BW also did not show significant differences between treatment means (P=0.975).

It can be concluded that probiotic supplementation in defaunated animals fed low-quality forage alone resulted in an increase in protozoal and bacterial counts (more than 30% and 40%, respectively), which was 5% higher when compared to natural refaunation of the rumen alone. Probiotic supplementation can also stabilize the pH of these animals fed low-quality forage alone with an increase of 12% from the defaunated values. Although there have been several debates focusing on the pros and cons of defaunation, more merits on the technique's pros were given. The effectiveness of probiotic supplementation in defaunated goats apparently depends on the type of diet given to the animal. This study has opened new doors for questions in defaunation-probiotic supplementation as a joint rumen manipulation strategy. Further studies can be done by controlling protozoal population while increasing bacterial population.

Figure 6. Effects of probiotic supplementation on DMI (g) of faunated versus defaunated goats.

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