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Nutrient Digestibility in Rabbits as Affected by Effective Microorganisms (EM), Yeast Culture and Enzymes

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ABSTRACT. *Forty eight, 9–10 weeks old rabbits (mean body weight of 2150 g) of both sexes were allotted to 8 treatments to study the effect of effective microorganisms (EM), yeast culture and commercially available enzymes (cellusase and chitinase) on crude protein (CP) and crude fibre (CF) digestibility. The experiment was carried out for 7 weeks at the University of Peradeniya Livestock Farm, Uda-Peradeniya. A low quality ration (LQR - containing 50% rice bran and 25% poultry by product meal - T1) or commercial broiler finisher ration (BF - T2) were fed (restricted) alone or in combination with above 3 additives (LQR+EM = T3; LQR+ yeast culture = T4; LQR+enzymes = T5; BF+EM = T6; BF+yeast culture = T7; BF+enzymes = T8). In addition to concentrates, dried* ***Brachiaria brizantha*** *grass and water were provided* ***adlibitum****. Rabbits were caged individually and were arranged in complete randomized design (CRD). Each treatment had 6 replicates. Individual feed intake was measured weekly. Nutrient digestibility values were estimated during 5th, 6th and 7th week of the trial. The CP and CF digestibility showed significant ($p<0.05$) differences among treatments. The highest CP digestibility was observed in BF+EM treatment (0.711) which was 1.86% higher than that of unsupplemented BF. But, LQR+yeast showed 10.5% higher CP digestibility (0.673) compared to that of unsupplemented LQR. Among CF digestibility values, LQR+enzyme gave the highest value (0.193) followed by LQR+EM (0.184) and LQR+yeast (0.179). Though the feed intake was highest in LQR+yeast, the highest weight gain was observed in LQR+enzyme.*

Present results clearly indicate that appropriate feed additives can improve nutrient digestibility in rabbits, especially when the quality of the diet is low. Out of the additives tested, enzymes have a greater effect on fibre digestibility.

INTRODUCTION

Domesticated rabbit (*Oryctolagus cuniculus*) has a great potential as a source of meat. It is an established fact that rabbits are good converters of fibrous plant material into lean meat and they certainly can tolerate a substantial fibre level in their diet (Cheeke, 1987). Lebas, 1984 have expressed that minimal fibre requirements, in terms of indigestible crude fibre content of the diet. However, being a monogastric animal, rabbits can utilize fibre as a result of hind gut fermentation which in fact is less efficient to rumen fermentation. Therefore, there is a great potential of using feed additives to enhance the digestibility of fibrous feeds in monogastric animals. Various experiments have

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demonstrated that supplemental probiotics and enzymes can increase the feed digestibility, nutrient utilization and therefore, the performance of chicken and pigs (Russo *et al.*, 1976;. Laksesvela, 1976; Feighner and Dashkevicz, 1988; Campbell, 1989; Mc Cracken *et al.*, 1993; Cowan *et al.*, 1996). However the use of such additives in rabbit diets is less investigated.

Probiotics, such as yeasts (Maertens and De Groote, 1992) and *Bacillus* (Mc Cartney, 1994) are gaining recognition in rabbit feeding. It is expected that these microorganisms are capable of colonizing the gut and contributing to the maintenance of the flora equilibrium (Maertens and De Groote, 1992). Some of them have been shown to benefit rabbit performance (De Blas *et al.*, 1991). Hollister *et al*,. (1990) indicated that probiotics significantly improve feed conversion and reduce enteritis incidence in weanling rabbits. Effective microorganisms (EM) may also be able to play an important role in monogastric feeding with their ability of degrading organic materials. Effective microorganisms can accelerate degradation and reduce carbon percentage in different organic materials of either high or low C/N ratio compared to those without EM.

Present study was carried out to investigate the effect of exogenous enzymes (cellulase and chitinase), yeast culture and effective microorganisms on nutrient digestibility in rabbits when supplemented to high and low quality diets.

MATERIALS AND METHODS

A feeding trial was conducted in the rabbit unit of University Livestock Farm, Uda-Peradeniya which is situated in mid country wet zone. All chemical analyses were done in the nutrition laboratory of the Department of Animal Science, University of Peradeniya.

Nine to ten weeks old, 48 rabbits with average body weight (BW) of 2150 g of both sexes were selected and housed in individual wire net cages having a floor space of 2160 cm^2 (72 cm × 30 cm) and height of 30.5 cm each. Cages were installed 61 cm above the ground level. Each cage had 2 separate feeders for concentrate (external) and roughage (internal) feeding. Cages were equipped with nipple drinkers. A nylon net was fixed under each cage for faeces collection. A broiler finisher ration (BF) was used as the high quality feed (positive control). A low quality ration (LQR) was formulated using 50% rice bran, 25% poultry by-product meal, 15% maize meal, 9.5% coconut poonac, 0.25% salt and 0.25% vitamin premix to contain similar crude protein(CP) levels (negative control). Another 6 dietary treatments were produced by supplementing each of 2 diets with enzymes (cellulases and chitinase) or yeast culture or EM, thus having 8 treatments in total. Enzymes (Roxazyme G and Alzyme) and yeast cultures (Sacchoromyces spp. in dry form) were added to concentrates at the rate of 200 ppm. In the EM treatment, EM culture (obtained from Sarvodaya) was sprayed on grass and added into water (as per recommendation given by the product) at the rates of 1:100 and 1:2000 respectively, immediately before feeding. Each dietary treatment was then assigned to 6 rabbits.

All rabbits, arranged in Completely Randomised Design (CRD) in 48 cages were fed with dried *Brachiaria brizantha* (*adlibitum*) and concentrate (55 g day^{-1}) in the morning

(8:00–11:00 am). Water was given *adlibitum* through nipple drinkers except for EM treated animals for which water was given in separate vessels after mixing with EM. After an adaptation period of 3 weeks and a preliminary period of 1 week, samples of faeces from individual rabbits were collected for 3 consecutive days starting from 31st, 38th and 45th days after commencement of the treatments. Soon after collection, the samples were stored in a deep freezer until they were taken for analysis. Feed (concentrates and grass) samples were also collected during each collection period and stored for subsequent analysis.

Feed intake was recorded weekly. Animals were weighed at the beginning and at the end of the experiment to determine changes in the body weight. Proximate analysis of collected feed and faeces samples was performed. Fifty gram of feed and faece samples were dried at 60°C for 48 h and ground separately. The samples were sieved through a 1 mm sieve, and thoroughly mixed samples were kept separately in airtight containers (labelled) until being used for analysis.

Proximate analysis on Dry Matter (DM), Ash, Crude Protein (CP), Crude Fibre (CF) and Ether Extract (EE) content of feeds was conducted using standard procedures (AOAC, 1985). Hundred and forty four (48×3) faeces samples were tested for DM, CP and CF. Gross energy of feed samples was determined by using a bomb calorie meter. Acid Insoluble Ash (AIA) concentration of feeds and faeces samples were determined using standard method (AOAC, 1985) as AIA was used as an internal indicator. Approximate digestibility values were calculated by using the following formula:

$$Apparent\ digestibility = 1 - [\frac{\%\ indicator\ of\ the\ feed}{\%\ indicator\ of\ the\ faeces} \times \frac{\%\ nutrient\ of\ the\ faeces}{\%\ nutrient\ of\ the\ feed}]$$

Statistical analysis

The digestibility (treatment and time were considered as two factors), weight gain and feed intake data were analysed according to CRD design, using the SAS package. Significant means were compared using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Nutrient composition of concentrates

The nutrient composition of concentrate mixtures given to the rabbits during the experimental period is given in the Table 1.

The dry matter percentages of 2 rations were within the accepted levels (89%). Although the CP requirement of adult rabbit is 13–16% (Lebas, 1980), these experimental diets contained higher levels of CP. However, consumption of grass must have diluted the CP levels in the total diet. Broiler Finisher ration contained a less amount of CF (5%) compared to the formulated Low Quality Ration (14%). Lebas (1980) has reported that, 14–16% C F is sufficient for rabbits. Rabbits that ate Broiler Finisher ration in this experiment had a free choice of eating enough roughage to balance their CF requirement.

Table 1. The nutrient composition of experimental diets (% on DM basis).

Nutrients	Concentrates	
	Low quality ration	Broiler finisher
Dry Matter (%)	89.70	89.91
Ash (%)	6.34	12.98
Crude Protein (%)	22.91	19.90
Crude Fibre (%)	4.98	14.28
Ether Extract (%)	7.99	10.12
NFE (%)*	57.78	42.72
Gross Energy (kcal kg^{-1})	5431	5200

* NFE - Nitrogen Free Extract (calculated value)

Because of high level of undefatted rice bran and PBM, the crude fat content of low quality ration was higher (10%) than in broiler finisher ration (8%). Low quality ration contained higher ash content (12%) and lower NFE than broiler finisher ration (6%). High ash content was due to high level of rice bran and PBM. It was assumed that the supplementation of diets with enzymes, yeast culture or effective microorganisms had no influence on the nutrient levels of diets.

The composition of roughage

The nutrient composition of dried *Brachiaria brizantha* fed to rabbits in the study is given in the Table 2. These 3 samples were collected during the 5th, 6th and 7th weeks of the feeding trial.

Table 2. Composition of *Brachiaria brizantha* (% on DM basis) of three collection periods (on 5th, 6th and 7th weeks) during the feeding trial.

Nutrients	Period 1	Period 2	Period 3	Mean
Ash (%)	8.23	8.70	8.05	8.32
Crude Protein (%)	9.40	7.90	9.79	9.03
Crude Fibre (%)	35.60	36.54	35.66	35.93
Ether Extract (%)	2.78	2.67	2.87	2.77
NFE (%)*	43.99	44.19	43.63	43.93
Gross Energy (kcal kg^{-1} dry matter)	4995	4965	5000	4986.70

* NFE - Nitrogen Free Extract (calculated value)

During the 2nd collection period, CP content was less than that of 1st and 3rd collection periods. However, the CF content was higher during 2nd collection period compared to 1st and 3rd collection periods. This may be due to the fact that the matured parts were harvested during 2nd collection period, because of unavailability of immature forages.

Feed intake

The mean concentrate intake of rabbits during the experimental period is shown in Fig. 1.

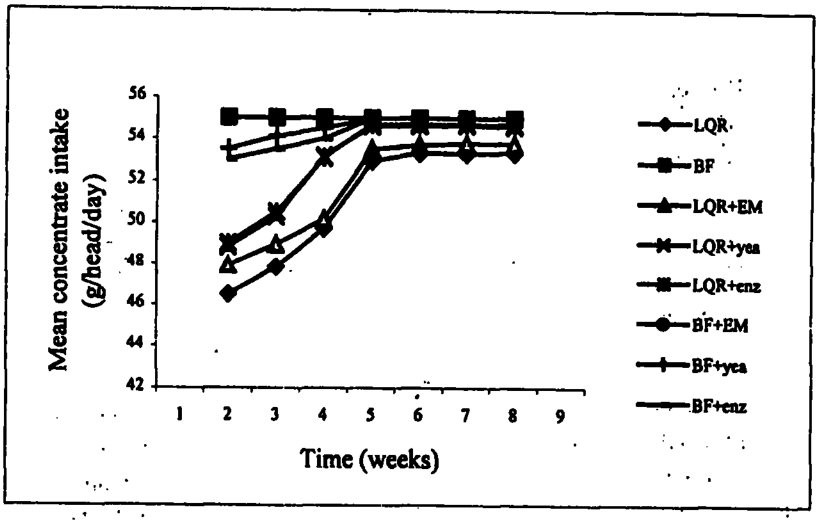

Fig. 1. Mean concentrate intake during experimental period (as fed basis).

Average concentrate intake and grass intake after the adaptation period is given in Table 3.

The rabbits took 3 weeks to adopt to the low quality rations. Therefore, all treatments containing LQR had lower consumption during 1st 3 weeks. It was mainly due to the stress caused by changing the normal feed. There was a gradual increase in the intake of formulated ration during preliminary period and then it was relatively constant. Most of the animals ate the whole amount of concentrates given to them except for LQR diets. The broiler finisher ration (T2) and other treatments that were mixed with additives (BF+EM, BF+yeast and BF+enzyme) did not show any significant ($p<0.05$) difference in concentrate intake. The LQR with or without additives showed less intake compared to the BF with or without additives. This may be due to the presence of high fibre content and poor quality protein in LQR. On the other hand, the additives (EM, yeast culture and enzyme) to LQR significantly ($p<0.05$) increased the concentrate intake compared to the unsupplemented LQR (T1).

Table 3. Mean concentrate and grass intake by rabbits as fed basis (g head^{-1} day^{-1}).

Treatments	Mean concentrate intake	Mean grass intake
LQR	53.35[a]	80.85[b]
LQR + EM	53.79[b]	83.72[e]
LQR + yeast	54.63[c]	84.15[f]
LQR + enzyme	54.71[cd]	81.31[c]
Broiler Finisher (BF)	55[d]	81.42[c]
BF + EM	55[d]	79.89[a]
BF + yeast	55[d]	81.87[d]
BF + enzyme	55[d]	81.27[c]

Means with different superscripts are significantly different ($p<0.05$).

The highest grass intake was observed when LQR was supplemented with yeast, that was followed by LQR+EM, BF+yeast, BF, LQR+enzyme, BF+enzyme, LQR and BF+EM treatments. The BF, LQR+enzyme and BF+enzyme treatments were not significantly ($p<0.05$) different. Lowest intake in BF+EM may be due to the highest intake of concentrate that must have reduced the appetite of animals or less preference towards grass, which were sprayed with EM solution. But in the case of LQR+ EM, grass intake was higher than other treatments except LQR+yeast treatment. As these animals ate fewer amounts of concentrate, it may have made them to eat more roughage. Lower values were also observed in the average concentrate intake of young rabbits fed with same diets (Barana *et al.*, 1999). This may be due to the effect of age.

Weight gain

The comparison of mean body weight gain of rabbits during the experimental period is given in the Table 4.

Weight gain values also indicated significant ($p<0.05$) difference among the treatments. Though LQR+yeast showed highest total dry matter intake, LQR+enzyme gave the highest weight gain. The significantly highest WG recorded in LQR+enzyme can be attributed to highest CF digestibility (Table 6) as well as comparatively higher concentrate intake (higher than LQR, LQR+EM and LQR+yeast). Bhatt *et al.*, (1996) also recorded

Table 4. Comparison of mean body weight gain of rabbits.

Treatments	T1	T2	T3	T4	T5	T6	T7	T8
Wt. gain g head^{-1} day^{-1}	8.1^a	10.18^e	9.3^c	8.51^b	12.2^h	11.5^g	10.7^f	10.1^d

Means with different superscripts are significantly different ($p<0.05$).

beneficial effects when an enzyme cocktail was added to diets for rabbits. Additives with BF increased the weight gain of rabbits except for BF+enzyme which (T8) was less than the unsupplemented BF (T2). But, Tor-Aghidye *et al.* (1992), Remais *et al.*, (1996) and Fernandez *et al.*, (1996) did not find any benefit from the inclusion of different enzymes in rabbit feeds. This may be due to various factors such as differences in diet, age, breed, sex, climatic factors *etc.* Barana *et al.* (1999) reported higher weight gain of rabbits than the present values. As the young growing rabbits were used, they must have shown better weight gain than that of present study.

CP digestibility

The CP digestibility values for 3 collection periods (during 5th, 6th and 7th weeks of experiment)by rabbits is given in the Table 5.

Table 5. Digestibility of crude protein of different diets during three collection periods (on 5th, 6th and 7th weeks) by rabbits.

Treatments	Period 1	Period 2	Period 3	Mean
LQR	0.59	0.59	0.64	0.609^a
LQR + EM	0.60	0.64	0.64	0.629^b
LQR + yeast	0.65	0.67	0.68	0.673^c
LQR + enzyme	0.62	0.63	0.63	0.630^b
BF	0.68	0.67	0.73	0.698^d
BF + EM	0.69	0.70	0.73	0.711^e
BF + yeast	0.68	0.68	0.73	0.696^d
BF + enzyme	0.59	0.60	0.67	0.697^d

Means with different superscripts are significantly different ($p<0.05$).

Crude protein digestibility increased with time. The additives improved CP digestibility of LQR. Among LQR treatments, the highest CP digestibility over the periods was recorded in LQR+yeast. According to Maertens and De Groote (1992), the probiotics (beneficial live or revivable microorganisms) are capable of colonizing the gut, contributing to the maintenance of the flora equilibrium. De Blas *et al.* (1991) also have noted better performances in rabbits when diets were added with probiotics. These records support the result of the present study. Beneficial effects of probiotics have been explained by Pollman (1986) as follows: a change in enteric flora and reduction of *Escherichia coli*; a decrease in intestinal pH; a production of antibiotic substances and a reduction of toxic amines and ammonia levels in the gastrointestinal tract and blood. These effects must have helped to increase the CP digestibility in LQR+yeast culture. However, addition of yeast with BF did not give better performance. Similar results have been reported by other researchers (Deshmkh and Pathak, 1991; Singh *et al.*, 1995). Barana *et al.* (1999) did not observe a significant effect when a similar LQR was supplemented with yeast. This may be due to the effect of age. On the other hand, no significant differences were observed in the CP digestibility when the BF was given with additives except for BF+EM which showed the highest CP digestibility. It is a well established fact that the additives like enzymes and probiotics will be effective if the diet is of poor quality. High quality diets are readily digested thus the additives have a less effect. This may be the reason for the above result. Similar results were recorded by Barana *et al.* (1999). However, the values were little higher than that of present study. The reason may be the age effect. According to Fraga (1998), the CP digestibility decreases following weaning. Addition of EM has enhanced the CP digestibility of LQR as well as of BF. Luther (1985) reported that the treatment of corn silage with a microbial inoculant increased the digestibility of both dry matter and CP.

Crude fibre digestibility

The crude fibre digestibility values during 3 collection periods and their means are given in Table 6.

Table 6. Digestibility of crude fibre of different diets during three collection periods (on 5th, 6th and 7th weeks) by rabbits.

Treatments	Period 1	Period 2	Period 3	Mean
LCR	0.164	0.164	0.165	0.164^{a}
LCR + EM	0.167	0.188	0.196	0.184^{de}
LCR + yeast	0.165	0.170	0.200	0.179^{cd}
LCR + enzyme	0.181	0.197	0.199	0.193^{e}
BF	0.164	0.171	0.172	0.169^{abc}
BF + EM	0.164	0.172	0.171	0.169^{abc}
BF + yeast	0.164	0.161	0.182	0.169^{abc}
BF + enzyme	0.173	0.170	0.181	0.175^{bcd}

* Means with the same superscripts are not significantly different ($p<0.05$).

Inadequate adaptation must have led to the lower CF digestibility values in period 1 and 2 compared to period 3. Fibre digestibility increases with age. Although, the CF digestibility values increased with time, the pattern was similar except in BF+yeast and BF+enzyme where the CF digestibility values were slightly low in the period 2. In LQR, the values were more or less constant during 3 periods and it was the lowest among 8 treatments. This may be because of higher CF content with the absence of additives in the diet. As the treatments LQR+EM, LQR+yeast and LQR+enzyme expressed significantly ($p<0.05$) higher CF digestibility values compared to LQR, beneficial effect of additives is apparent when added with LQR. Among the mean values, LQR+enzyme was the highest. Addition of enzyme with BF also showed significant ($p<0.05$) difference from BF (T2). Increased fibre digestibility in monogastric animals when their diets were supplemented with cellulase enzyme was reported by many authors (Samarasinghe and Wenk, 1993; Wenk *et al.*, 1993) thus supporting the present results. The LQR+EM did not differ significantly ($p<0.05$) from LQR+enzyme, and LQR+yeast did not significantly ($p<0.05$) differ from LQR+EM. Beneficial effects of probiotics (stated under CP digestibility) may be the reason for better performance of LQR+enzyme. Addition of EM to LQR has improved the CF digestibility. It is in agreement with the results of Cleale (1990), who also found that the digestibility of DM and acid detergent fibre (ADF) in corn silage was increased with the addition of a microbial inoculant. Recent studies have shown that the application of certain microbial inoculants during ensilage can enhance the fermentation process and thereby results in the improvement in silage quality as evidenced by increased consumption, digestibility and animal performance (Mayne, 1990; Smith, 1993).

Additives, except enzymes did not express significant improvement when added to BF. Since BF contained very less amount of CF (4.9%) compared to LQR (14.2%), the endogenous enzymes and microbes present in the gut itself must have digested the diet efficiently. Therefore, the additives may have not played a vital role in feed digestibility when added to BF.

CONCLUSIONS

Effective microorganisms, yeast and appropriate enzymes increase the nutrient digestibility in rabbit feeds. The effects are more prominent when the quality of the diet is low. These findings indicate that low quality feeds can be efficiently fed to rabbits using appropriate additives in order to raise rabbits more economically.

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