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*Original Contribution*

# EFFECT OF THE MULTIENZYME PROTOZIN ON VOLATILE FATTY ACIDS LEVEL IN THE DIGESTIVE SYSTEM OF RABBITS

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**ABSTRACT**

Six month-old male rabbits of the White New Zealand breed, divided in two groups, Control and Test, were used for the experiment. The fundus of the stomach and the proximal part of the caecum of these animals were cannulated. Then each animal was reared in a cell (40/50 cm) of a three-layer cell battery in a closed room with regulated temperature and air humidity. After a 10-day post operative period, the multienzyme *Protozin* was added, at a dose 1g/kg of concentrate forage, to the mixture concentrate that served as food for the animals. The study started after a week of adaptation to the new diet. Our results showed the following: at *Protozin* concentration of 1g/kg of concentrate forage there was a significant increase in stomach VFA before and after feeding ($p<0.001$; $p<0.05$). On the other hand, *Protozin* did not bring about any significant change in caecum VFA. However, it increased the molar concentration of acetic acid before and after feeding ($p<0.05$).

**Key words:** Rabbits, VFA and Food Degradability

## INTRODUCTION

The home rabbit is a single-stomach animal. However, its digestive system has a number of peculiarities that make it distinct, by type of food degradability, from the other single stomach animals. It has well-developed actively functioning caecum The single chamber stomach is well-developed and a high percentage of the proteins in the diet are decomposed in it. An important feature of digestion in rabbits is the presence of faecafagia – a process that is extremely important for the efficient utilization of nutritive substances, some microelements and vitamins.

The digestion in the rabbit caecum is accomplished under the influence of enzymes from the gastric, pancreatic juice and the caecum microflora. The presence of microorganisms and extracellular microbe enzymes makes the digestive processes in the caecum similar to those in the fore-stomachs of ruminants. In addition, the metabolic activity of microorganisms in the caecum provides an important source of protein and carbohydrate supplements in the rabbit. The parameters that determine the caecum medium (osmotic pressure, acidity, etc.) are crucial for the activity and the size of microorganism populations. These parameters can be modified by some changes in the content of the diet or through the supplementation of biologically active substances to improve the caecum medium. According to Kurilov *et al*. (1), the presence of VFA and lactic acid in the rabbit stomach influences microbial degradability due to the faecafagia. Entry of microorganisms into the stomach depends on this. In the stomach the mucous envelope of the soft feces stays for not more than 6 hours and during that time pH in it is about 6.4, and this is favorable for the survival of microorganisms and for some fermentation processes to take place.

The pH of the stomach content with the hydrochloric acid concentration also influences the activity of the stomach microflora. According to Naumova (2) the uneven distribution in the main chamber and cell wall of the various zones of the stomach mucosa is of crucial importance for the viability and metabolic activity of microorganisms in the stomach. For example, most of the microbe enzyme-catalyzed catabolic processes take place in the cardiac area of the stomach. Any situation that could alter this situation would undoubtedly affect the integrity and entire gut function of the rabbit.

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The objective of this study, then, is to establish the effect of the multienzyme *Protozin* on the total amount and molar proportion of VFA in the stomach and caecum of rabbits.

## MATERIAL AND METHODS

Six male rabbits of the White New Zealand breed aged 6 months were used in the study. They were divided in two groups: Control and Test. The fundus of the stomach and the proximal part of the caecum (widest part of the intestine) were cannulated. The tests began ten days later when recovery from the cannula wounds must have occurred.

The animals were reared all the while in individual cells, measuring 40/50 cm, of a three-layer cell battery in a temperature and air humidity-regulated room. Each animal was fed food and water *ad libitum* twice in a day: at 8 a.m. after taking the samples and at 1.00 p.m. **Table 1** shows the constituents of the diet provided. The nutritive value of the diet is given in **Table 2**.

**Table 1.** Chemical contents of the diet

| Components | % at laboratory moisture | | | | In % of DM | |
|---|---|---|---|---|---|---|
| | Water | DM | Crude Protein | Crude Fiber | Crude Protein | Crude Fiber |
| Concentrated forage | 10.84 | 89.16 | 11.63 | 5.16 | 13.04 | 5.79 |
| Alfa alfa hay | 8.77 | 91.23 | 15.86 | 31.29 | 17.38 | 34.29 |

Record of daily consumption was, however, kept. Ten days after cannulation the multienzyme *Protozin*, at dose 1g/kg of concentrate forage, was added to the mixture concentrate that served as food for the animals. The tests began after a week of adaptation by the experimental animals to the diet and the enzyme product.

Protozin is manufactured by *Plastchim*, Botevgrad. According to the manufacturer protozin is a substance with a wide range of enzyme activities comprising the following constituent enzymes: amylase, cellulase, xylanase, β-glucanase, protease, lipase, phytase, as well as a nutrient medium for the enzymes. Protozin improves food degradability and increases assimilation of the basic nutrients, which are, starch, carbohydrates, proteins, fats and cellulose from 6 to 15% and increases the growth rate to 10%. The product increases the caloric value of the diet by 50-60 Kcal/kg of forage and reduces the cost of the end product from the fattening of the animals. The product is recommended for young animals with relatively underdeveloped digestive enzyme activity. It is effective in a wide range of pH. The total amount of VFA in the stomach and caecum of the animals was obtained using steam distillation in Marcham's apparatus according to Krotkova and Mitin's (3) method. This was then followed by molar correlation of VFA in the stomach and caecum using a gas chromatograph "Carlo Erba" according to Kellogg's method (4).

Test samples were all the time taken from the cannulas inserted in the stomachs and intestines of these animals on three successive days: in the morning before feeding and at noon, that is, five hours after feeding.

## RESULTS AND DISCUSSION

### Total amount and molar concentration of VFA in the stomachs of the experimental animals

**Table 2** presents the data on the molar concentration of the acetic and butyric acids in the stomachs of the experimental animals from both groups.

In spite of the low level of fermentation processes in the stomachs of rabbits volatile fatty acids were present at about 1-2.5 mmol/100ml concentration (5). For comparison the level of VFA in the fore-stomachs of ruminants is about 9 mmol/100ml.

It is suggested that this relatively low concentration of VFA in the stomach could be related to the energy supply of the organism. It has already been explained that the high concentration of hydrogen ions in the stomach does not create favorable environment for fermenting the complex carbohydrates in the forages. Nevertheless, fermentation processes take place in the stomach of rabbits and this is the beginning of fiber degradability.

**Table 2** presents the data on total amount and molar correlation of VFA in the stomachs of the experimental animals.

**Table 2.** Total amount /mmol/100ml/ and molar concentration /mol %/ of VFA in the stomachs of the experimental animals

| Parameters | n | Before feeding | | Five hours after feeding | |
|---|---|---|---|---|---|
| | | x ± Sx | C | x ± Sx | C |
| | | | Protozin | | |
| Total amount of VFA | 6 | 3,01 ± 0,23 *** | 19,88 | 2,38 ± 0,32 * | 40,15 |
| Acetic acid C2 /mol %/ | 6 | 83.43 ± 2.07** | 3.00 | 88.96 ±1.97 | 3.02 |
| Butyric acid C4 /mol %/ | 6 | 14.16 ± 1.58* | 3.00 | 9.16 ±1.29 | 3.01 |
| | | | Control | | |
| Total amount of VFA | 6 | 1,74 ± 0,23 | 40,09 | 1,36 ± 0,19 | 40,14 |
| Acetic acid C2 /mol %/ | 6 | 89.02 ± 2.24 | 2.23 | 10.08 ±1.79 | 2.00 |
| Butyric acid C4 /mol %/ | 6 | 9.8 ±0.07 | 2.44 | 10.08 ±1.79 | 2.00 |

**** p<0.001 ; * p<0.05 – control versus experimental group*

According to Naumova (2) the uneven distribution of the main chamber and cell walls in the various zones of the stomach mucosa is extremely important for the viability and the metabolic activity of the microorganisms in the stomach. And the cardiac area of the stomach presents the best environment for the microbe enzyme-catalyzed catabolic processes. When the food mass moves towards the fundus and the pylorus, the concentration of the hydrochloric acid increases thereby causing the death of the microorganisms. According to the same author the greatest number of dead microorganisms can be isolated from stomach content taken from the small curve of the stomach.

Apart from the metabolic activity of microorganisms the level of VFA in the stomach can also be considered within the context of their resorption through the stomach wall.

After addition of the studied enzyme the level of VFA in the stomach increased significantly ($p<0.001$; $p< 0.05$). This could have been due to the increased cellulose-digestion processes in that group of animals studied. The values before and after feeding were 3.01 mmol/100ml and 2.38 mmol/100ml, respectively, and for the Control they were 1,74 mmol/100 ml and 1.36 mmol/100 ml.

**Total amount and molar concentration of VFA in the caecum of the experiemntal animals**

The data on the level of VFA in the caecum of the experimental animals are presented in **Table 3**. The values before feeding for the experimental and the Control groups were 13.64 mmol/100 ml and 14.87 mmol/100 ml, respectively. The insignificantly low values after feeding in the Control group could have been due to the accelerated resorption of VFA through the intestinal wall.

There was a significantly higher level of acetic acid in the experimental group before feeding. The values for the Control and the experimental groups were 70.20 mol % and 79.40 mol %, respectively. The butyric acid levels followed a similar trend in the two groups, recording 16.58 mol % and 14.83 mol % in the experimental and Control, respectively. According to Kalugin (5) the molar correlation of VFA in the caecum of rabbits is 66 - 84 % acetic acid, 6 - 16 % propionic acid, 8 - 25 % - butyric acid.

The propionic acid level in the experimental group did not show any significant difference over that of the Control, at time before feeding. It was 5.78 mol % and this has significant importance for fattening animals. In the same group there was an insignificant drop in the level of butyric acid 5 hours after feeding compared to what obtained at time running up to feeding and the values were 15.21 mol % and 16.58 mol %, respectively. The differences in the molar correlation of the VFA in the caecum are presented in **Table 3**.

The pH values in the caecum depended, to a certain extent, on the level of VFA in the caecum. According to Samoylenko (6), pH in the caecum varies in the range 6.0-7.4 and depends on the type of the diet, the level of degradability of nutrients in the stomach and small intestines, the caecum wall movement and the level of the carbohydrate atoms. The constantly incoming alkaline hymus from the small intestines to the caecum maintains pH, which is close to neutral and creates favorable conditions for the development of the microorganisms in the caecum.

We should emphasize here that the total amount of VFA in the caecum of the experimental animals was considerably higher than that in the stomach content. The same fact is valid for the molar concentration of VFA as well. These data would be useful in determining the considerable role of the

caecum in rabbits with regard to degradability of crude fiber and complex carbohydrates in the diet and the energy supply of the organism in these animals.

**Table 3.** Total amount /mmol/100ml/ and molar concentration /mol %/ of VFA in the cecum of the experimental animals

| Parameters | n | Before feeding | | Five hours after feeding | |
|---|---|---|---|---|---|
| | | x ± Sx | C | x ± Sx | C |
| | | | Protozin | | |
| Total amount of VFA | 6 | 13.64 ± 1.17 | 25.67 | 13.82 ± 1.05 | 22.71 |
| Acetic acid C2 /mol %/ | 7 | 79,40 ± 2,56 * | 8,54 | 79,01 ± 2,85 | 10,21 |
| Propionic acid C3 /mol %/ | 7 | 3,89 ± 2,38 | 161,63 | 5,78 ± 2,07 | 101,44 |
| Isobutyric acid C4 I /mol %/ | 7 | 0 | 0 | 0 | 0 |
| Butyric acid C4 /mol %/ | 7 | 16,58 ± 2,60 | 41,42 | 15,21 ± 2,02 | 37,52 |
| | | | Control | | |
| Total amount of VFA | 6 | 14.87 ± 1.24 | 20.42 | 13.87 ± 0.55 | 11.28 |
| Acetic acid C2 /mol %/ | 7 | 70,20 ±2,48 | 9,34 | 78,39 ± 3,49 | 11,79 |
| Propionic acid C3 /mol %/ | 7 | 14,79 ± 4,62 | 82,70 | 5,13 ± 2,63 | 135,55 |
| Isobutyric acid C4 I /mol %/ | 7 | 0 | 0 | 0 | 0 |
| Butyric acid C4 /mol %/ | 7 | 14,83 ± 2,96 | 52,86 | 16,48 ± 1,79 | 28,79 |

*$p<0.05$ - control versus experimental group*

## CONCLUSIONS

Our results have shown that the multienzyme Protozin has the following effect on the level of VFA in the digestive system of rabbits:

1. At dose 1g/kg of the animal's diet protozin caused some significant increase in the level of VFA in the stomach of animals in the experimental group before and after feeding ($p<0.001$; $p<0.05$).
2. Adding protozin to the diet of rabbits resulted in no significant changes in the total amount of VFA in the caecum but, however, led to increase in molar concentration of the acetic acid before and after feeding in the experimental animals ($p<0.05$).

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