Effect of *Kemzyme* - Bentonite Co-supplementation on Cecal Fermentation and Metabolic Pattern in Rabbit

M. A. Abdl-Rahman (Corresponding author)
Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Egypt
Faculty of Veterinary Medicine, Cairo University, Giza-12211, Egypt
Tel: 202-2864-5669 E-mail: mahmod.abdelhafez@gmail.com

F. A. R. Sawiress
Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Egypt
Faculty of Veterinary Medicine, Cairo University, Giza-12211, Egypt
Tel: 202-2391-5546 E-mail: francoisawiress@hotmail.com

Sohair Y. Saleh
Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Egypt
Faculty of Veterinary Medicine, Cairo University, Giza-12211, Egypt
Tel: 202-3338-6209 E-mail: sohair saleh@hotmail.com

Abstract

With the objective of improving the impact of multi-enzymes feed additives on cecal fermentation pattern and rabbit metabolism, sodium bentonite was co-supplemented with *"Kemzyme",* a multi-enzyme blend of *Kemin Agrifoods Europe.* Co-supplementation decreased cecal pH value, increased total VFAs concentration, increased propionate at the expence of acetate and butyrate, increased fermentation efficieny and VFAs utilization. Additionally, co-supplementation increased serum glucose concentration and decreased serum triglycerides and cholesterol concentrations. Cecal ammonia nitrogen and serum urea concentration were also decreased by co-supplementation while no change was recorded in serum total proteins concentration. The study therefore, suggested that, coupling bentonite to multi-enzyme feed additives could improve the impact of such enzymes on cecal fermentation pattern and rabbit metablism.

Keywords: Bentonite, Cecal fermentation, Feed additives, Multi-enzymes, Rabbit

1. Introduction

Being a small non-ruminant herbivore, rabbit feeding is more similar to ruminant feeding than to poultry feeding as rabbit digestive physiology shows some similarity to ruminants, particularly cecal processes (Marounek *et al*. 2000). Fermentation pattern in rabbit cecum resembles that in the rumen; however it shows lower fibrolytic microbial activity and relatively higher amylolytic and proteolytic microbial activity (Gidenne 1997). Feed cost represents 60-70% of rabbit rearing costs (Makkar *et al*. 1990) and, as a consequence, maximizing utilization of nutrients is essential to the profitability and sustainability of rabbit production. Several studies have been attempted for incorporating exogenous enzymes into rabbit diets to improve nutrients availability, however in most trials, rabbits appeared less responsive (Falcao-e-Cunha *et al*. 2007). It appears that the conditions (pH and temperature) through which these enzymes act are critical for their activity (Colombatto *et al*. 2003).

Mista (2007) suggested a decisive impact of bentonite on the pH reaction of rabbit cecum that was favorable on the bacterial processes that take place there. Because of its huge surface area and the negative charges on its surface, bentonite has a great ability to adsorb ammonia from a solution when the concentration of ammonia is high, and to release it when the concentration falls (Saleh 1994).

This study has been aimed at exploring whether coupling sodium bentonite to exogenous enzymes could improve their impact on cecal fermentation and rabbit metablism or not.

2. Materials and methods

The present study was performed at Department of Physiology, Faculty of Veterinary Medicine, Cairo

University.

2.1 Animals, diets and experimental design

Twenty rabbits of the Newzealand white breed, 12-week old and approximately 1.2 kg body weight were equally and randomly divided into four groups (5 in each). All rabbits received *ad libitum* a balanced commercial pellet diet (composition and chemical analysis are shown in table, 1). Group 1 kept untreated and served as a control. Group 2 (kemzyme group) was supplemented with 0.1% *"Kemzyme",* a multi-enzyme blend of *Kemin Agrifoods Europe,* composed of 3000 μ/g beta-glucanase, 5000 μ/g cellulase, 450 μ/g alfa-amylase and 450 μ/g protease. Group 3 (Bent group) was supplemented with 2% sodium bentonite while group 4 (Enz+Bent group) was supplemented with 0.1% *Kemzyme* plus 2% sodium bentonite. Doses of supplemented additives were given mixed with the basal ration in the form of pellets. The experiment lasted for 12 weeks during which fresh, clean water was available at all times.

2.2 Sampling procedures

At the end of the experiment, all rabbits were slaughtered by severing the jugular vein. Blood samples were collected into clean, centrifuge tubes and centrifuged at 3,000 x *g* for 10 minutes. Sera were separated and kept at –20 ^{o}C until assayed for determination of serum total proteins, urea, glucose, triglycerides and cholesterol concentrations.

The carcasses were subsequently opened, the ceca were removed and their contents were squeezed out into beakers. Immediately cecal contents were strained through two layers of sterile gauze and the resultant strained liquors were used for measuring pH values by electronic digital pH meter. Thereafter, the contents were centrifuged at 7,000 x *g* for 10 minutes. The supernatant fluid was divided into two parts. One part was acidified with 0.2 M hydrochloric acid solution (one ml-ml^{-1}sample) to be used for determination of ammonia nitrogen (NH_3-N) concentration while the other was treated with a solution of 5% orthophosphoric acid (v/v) plus 1% mercuric chloride (w/v) (0.1 ml-ml^{-1} sample) for determination of total VFAs concentrations and individual VFAs proportions.

2.3 Analytical Procedures for Samples of Cecal Digesta

Cecal NH_3-N concentrations were measured by spectrophotometry according to Chaney and Marbach (1962). Total VFAs concentrations were measured by steam distillation according to Eadie *et al*. (1967). The percentage concentrations of VFAs were analyzed using High Performance Liquid Chromatography (HPLC; Model Water 600; UV detector, Millipore Crop.) according to the method of Mathew *et al*. (1997). After the results of the percentage concentrations of the particular VFAs had been received, the following fermentation parameters were calculated:

2.3.1 The concentrations (mmol·l^{-1}) of acetic, propionic, butyric and valeric acids in their total concentration.

2.3.2 The fermentation efficiency (FE): This was calculated on the basis of the equation worked out by Orskov (1975) and modified by Baran and Zitnan (2002):

$$FE = (0.622a + 1.092p + 1.56b)\ 100/(a + p + 2b)$$

where: a, p and b express the concentrations (mmol·l^{-1}) of acetic, propionic and butyric acids respectively in the total concentration of VFAs produced. The final result of this equation is expressed in percentage and shows an amount of energy stored in VFAs as a percentage participation of the initial energy.

2.3.3 The VFAs utilization index: This was expressed by non-glucogenic VFAs/glucogenic VFAs ratio (NGGR) according to Orskov (1975):

$$NGGR = (A + 2B + V) / (P+V)$$

where A, P, B and V express the concentrations (mmol·l^{-1}) of acetic, propionic, butyric and valeric acids respectively. Valeric acid is classified as both glucogenic and non-glucogenic VFA because, its oxidation creates 1 mole of acetic acid and 1 mole of the propionic acid.

2.4 Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) test according to Snedecor and Cochran (1980). Treatment means were compared by the least significance difference (LSD) at 5% level of probability.

3. Results and discussion

Compared to other farm animals, the amount of research on rabbit feed additives is limited. Most of the published works have dealt with growth performances, much less with mechanisms of action, cecal fermenation pattern and metabolic parameters. Also, the impact of combinations of two or more of these additives received little attention.

3.1 Cecal fermentation traits

The results presented in table (2) reveal that, total VFAs concentrations were increased in all experimental groups in comparison with control group values. However, Enz+Bent co-supplementation achieved 3.6 mmol·l^{-1} additional increase in VFAs concentrations over the 10.6 mmol·l^{-1} increased by enzyme supplementation. According to Garcia *et al*. (2002), VFAs concentrations could reach a value of up to 99.8 mmol·l^{-1}, depending on rabbit's age and physiological status as well as food ingredients. For this study, it appears that fermentation within the cecum proceeded normally after co-supplementation as VFAs concentrations amounted to 75.8 mmol·l^{-1}.

Meanwhile, each treatment modified the VFAs profile within the cecum in a particular fashion. As for propionic acid, both Enz and Enz+Bent supplementations increased its proportions either at the expense of butyrate only (in case of enzyme-supplementation) or at the expense of acetate and butyrate (in case of co-supplementation). This acid is a valuable substrate in glucose synthesis in many species (Bergman, 1990) as it contributes in gluconeogenesis and formation of long-chain fatty acids in the liver and intermediate products of its changes participate in regulation of a series of processes, including ketogenesis, gluconeogenesis and ureogenesis (Remesy *et al*., 1995). Thus, increased propionate production in rabbit cecum seems to be favorable. Conversely, the high yield of acetates at the expense of propionates accompanying bentonite supplementation could be the end result of enhanced acetogenesis. Reductive acetogenesis (microbial synthesis of acetate from CO_2 and H_2) is a feature of rabbit cecal fermentation which, with age, is partly replaced with methanogenesis (Piattoni *et al*., 1996). Acetates participate in lipogenesis, cholesterologenesis and activate gluconeogenesis from lactate and pyruvate (Remesy *et al*., 1995). Additionally, bentonite supplementation was associated with increased butyrate, an essential precursors in lipogenesis (Remesy *et al*., 1995). According to Murray *et al*. (1990), an influence of bentonite on VFAs molar proportions in sheep was similar to the one obtained in this research when they were fed on concentrates. However, Galyean and Chabot (1981) did not find any significant changes in the VFAs profile of bull rumen under the influence of a mineral mixture containing sodium bentonite. Molar proportions of valeric acid showed no change except for Enz+Bent supplementation where they were increased in contrast to control.

Concerning NH_3-N concentrations, both Bent and Enz+Bent supplementations induced a decremental effect with reference to control. According to Macfarlane and Gibson (1995) a series of factors could influence NH_3-N concentrations within the cecum, including H_2 pressure, chyme reaction, and carbohydrates availability. In comparison with ruminants, proteolytic activity in the rabbit cecum is relatively higher (Gidenne, 1997) and ammonia levels fluctuate between 1.86–23.9 mmol·l^{-1} as shown by Garcia *et al* (2002). The tendency to lower NH_3-N concentrations observed by both Bent supplementation and Enz+Bent co-supplementation could be attributed to the great ability of bentonite to absorb ammonia. Ivan *et al* (1992) speculated that, bentonite could also absorb some proportions of proteolytic enzymes, which would then be unable to act on the dietary proteins or it could absorb free dietary amino acids that also would not be accessible to bacterial fermentation. These results are in agreement with those reported in ruminants by Wallace and Newbold (1991) and Saleh (1994) who found that, addition of bentonite led to a decrease in ruminal NH_3-N concentration. Furthermore, all supplementations decreased the cecal pH values, with the lowest values recorded in Bent and Enz+Bent groups. pH value of rabbit cecal chyme shows a falling tendency when VFAs concentrations grows and ammonia concentration falls (Garcia *et al* 2002), and hence, reduction of pH associated with Bent and Enz+Bent groups corresponded well with VFAs and NH_3 concentrations. These results are in agreement with those of Mista (2007) in rabbit and those of Murray *et al*. (1990) in sheep, that suggest an influential impact of bentonite on pH reaction of rabbit cecal contents.

It is evident from table (3) that, both Enz and Enz+Bent co-supplementation were associated with an improvements in fermentation effeciency and VFAs utilization, whereas, bent-supplementation exerted a damping effect on both. However, the value for co-supplementation was higher than that for Enz-supplementation by about 1% and higher than that calculated for control by about 2%. Calculation of fermentation efficiency is based on convertion of hexose energy to VFAs energy on the basis of equations worked out by Orskov (1975) and modified by Baran and Zitnan (2002). Metabolism of VFAs is less efficient than metabolism of glucose as more energy is used for formation of one mole of ATP (Livesey and Elia, 1995). It is clear that increased fermentation efficiency achieved by Enz+Bent co-supplementation is actually the end result of its ability to increase propionate a the expense of acetate and butyrate. In contrast to fermentation efficiency, higher values of VFAs utilization index indicate the inferior use of VFA (Czerkawski, 1986). The lowest value of NGGR, which indicates the most excellent utilization of VFA, was attained by Enz+Bent co-supplementation that achieved 36.5% improvement in VFAs utilization versus 25.3% for supplementing enzymes alone. Improvements achieved in fermentation effeciency, VFAs profile and VFAs utilization by

Enz+Bent co-supplementation could be attributed to optimization of cecal reaction for enhancing the impact of the added enzymes on cecal fermentation pattern

3.2 Blood biochemical parameters

Data presented in table (4) identify that, serum glucose concentrations were increased by both Enz and Enz+Bent supplementation, whereas, were decreased by Bent supplementation when compared to values of the control group. Increased serum glucose levels in both Enz and Enz +Bent groups could be attributed to increased propionate production in the cecum and enhanced propionate metabolism by the liver. Elliot (1980) suggested that, dietary changes greatly affect glucose production by gluconeogenesis in the liver via their effect on propionyl Co.A carboxylase activity, an enzyme essential in the biochemical pathway through which propionate is normally incorporated in the tricarboxylic acid cycle. Glucose reduction observed with bentonite supplementation corresponded well with values of propionates, yet it is likely that, high butyrate associated with bentonite supplementation additionally inhibited gluconeogenesis (Remesy *et al*., 1995).

both serum triglycerides and cholesterol concentrations were increased by Bent- supplementation and were decreased by Enz+Bent co-supplementation while, were not affected by Enz-supplementation. Increased serum triglycerides levels associated with bentonite supplementation could be attributed to increased butyrate, a valuable substrate for lipogenesis (Remesy *et al*., 1995), whereas, increased cholesterol concentrations is probably due to increased formation of chylomicrons required for cholesterol absorbtion from small intestine (Grummer & Carroll, 1991).

The recorded values for serum total proteins were within the normal ranges reported by Jones (1975), however, in all experimental groups, plasma proteins did not differ with reference to control group values. Plasma total proteins are mainly synthesized in the liver and these values indicate that the experimental animals were in a good nutritional status and liver has no pathological lesions.

Concerning serum urea concentration, it is evident from the results that urea concentrations followed a pattern simillar to that of cecal ammonia nitrogen concentrations where, both Bent supplementation and Enz+Bent co-supplementation resulted in significant decrease in urea concentrations. This is mostly due to little ammonia absorption and detoxification in the liver which means that co-supplementation had synchronized the rate of ammonia utilization to the rate of ammonia production. Leonard *et al* (1977) suggested that, hepatic detoxification of ammonia into urea could reduce plasma glucose level possibly by its direct inhibitory effect on liver gluconeogenetic activity through a competition for ATP by increased activity of urea cycle. Furthermore, propionate inhibit the synthesis of N-acetyl glutamate, the activator of carbamoyl phosphate synthetase which catalyses the major rate-limiting step in urea cycle (Choung & Chamberlain, 1995).

4. Conclusion

Results from this study suggest that, coupling bentonite to multi-enzyme feed additives could lead to favorable modifications in cecal environment presumably, acidification of cecal contents and stabilization of ammonia nitrogen concentrations. These alterations should be considered as an advantage, as they improve the impact of the supplemented enzymes on cecal fermentation pattern and rabbit metabolism.

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Table 1. Composition and chemical analysis of the used basal diet

Ingredients	% as fed	Chemical analysis	% of dry matter
Yellow corn	25.00	Crude fibers	14.00
Berseem hay	31.40	Crude proteins	17.50
Wheat bran	26.20	Ether extract	2.70
Soybean meal	14.00	Nitrogen free extract	58.00
molasses	3.00	total ash	7.80
Vitamin and mineral premix	0.30	Digestible energy (kcal/kg diet)	2200
DL-Methionine	0.10		

Table 2. Effect of Kemzyme and/or Bentonite supplementations on cecal fermentation pattern

Group / Parameter	Control	Kemzyme	Bentonite	Kemzyme +Bentonite	L.S.D
P^H value	$5.32^{a} \pm 0.02$	$5.24^{b} \pm 0.01$	$5.16^{c} \pm 0.01$	$5.15^{c} \pm 0.01$	0.03
Total VFAs ($mmol \cdot l^{-1}$)	$61.54^{a} \pm 0.52$	$72.19^{b} \pm 0.94$	$75.94^{c} \pm 0.21$	$75.80^{c} \pm 0.49$	1.79
Acetic acid (mole%)	$54.76^{a} \pm 0.48$	$55.45^{a} \pm 0.48$	$58.56^{b} \pm 0.41$	$52.28^{c} \pm 0.47$	1.38
Propionic acid (mole%)	$19.40^{a} \pm 0.82$	$23.99^{b} \pm 0.67$	$11.36^{c} \pm 0.36$	$26.18^{d} \pm 0.75$	2.02
Butyric acid (mole%)	$23.04^{a} \pm 0.48$	$17.78^{b} \pm 0.44$	$27.39^{c} \pm 0.64$	$17.13^{b} \pm 0.84$	1.85
Valeric acid (mole%)	$2.79^{a} \pm 0.20$	$2.79^{a} \pm 0.23$	$2.70^{a} \pm 0.22$	$4.41^{b} \pm 0.31$	0.73
NH_3-N ($mmol \cdot l^{-1}$)	$22.16^{a} \pm 0.68$	$21.32^{a} \pm 0.86$	$14.11^{b} \pm 0.78$	$15.98^{b} \pm 0.78$	2.32

Data presented as means ± SE, N =5

Values having different letters in the same raw are significantly different at P < 0.05

Table 3. Effect of Kemzyme and/or Bentonite supplementations on fermentation efficiency and NGGR

Group / Parameter	Control	Kemzyme	Bentonite	Kemzyme +Bentonite	L.S.D
fermentation efficiency (%)	$75.84^{a} \pm 0.26$	$76.93^{b} \pm 0.23$	$73.37^{c} \pm 0.12$	$77.91^{d} \pm 0.24$	0.66
NGGR	$4.70^{a} \pm 0.28$	$3.51^{b} \pm 0.14$	$8.28^{c} \pm 0.26$	$2.98^{b} \pm 0.13$	0.63

Data presented as means ± SE, N =5

Values having different letters in the same raw are significantly different at P < 0.05

Table 4. Effect of Kemzyme and/or Bentonite supplementations on some related serum biochemical indicators

Group / Parameter	Control	Kemzyme	Bentonite	Kemzyme +Bentonite	L.S.D
Glucose ($mmol \cdot l^{-1}$)	$4.20^{a} \pm 0.04$	$4.55^{b} \pm 0.04$	$3.97^{c} \pm 0.03$	$5.20^{d} \pm 0.02$	0.12
Cholesterol ($mmol \cdot l^{-1}$)	$1.48^{a} \pm 0.01$	$1.43^{a} \pm 0.01$	$2.14^{b} \pm 0.02$	$1.26^{c} \pm 0.02$	0.06
Triglycerides ($mmol \cdot l^{-1}$)	$0.74^{a} \pm 0.01$	$0.76^{a} \pm 0.02$	$0.99^{b} \pm 0.01$	$0.54^{c} \pm 0.01$	0.03
Total protein ($mmol \cdot l^{-1}$)	58.76 ± 0.39	58.44 ± 0.39	57.50 ± 0.69	58.14 ± 0.48	NS
Urea ($mmol \cdot l^{-1}$)	$9.33^{a} \pm 0.14$	$9.16^{a} \pm 0.14$	$7.18^{b} \pm 0.17$	$6.69^{b} \pm 0.21$	0.51

Data presented as means ± SE, N =5

Values having different letters in the same raw are significantly different at P < 0.05