Journal of Applied Sciences

ISSN 1812-5654

science alert

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Journal of Applied Sciences 15 (3): 610-612, 2015
ISSN 1812-5654

ansinet
Asian Network for Scientific Information

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/jas.2015.610.612

Influence of Substrates on Toxicity of Ruminal Fluid of Cattle

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ARTICLE INFO
Article History:
Received: November 11, 2014
Accepted: December 20, 2014

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ABSTRACT
Effects of various metal cations and physical methods of feed processing with different intervals of exposition of ruminal fluid with feed factor were assessed in the experiment by method of biotesting. Recombinant *E. coli* strain with cloned luxCDABE-genes *Photobacterium leiognathi*, ruminal fluid of beef cattle of Kazakh white-headed breed, model ruminal fluid and trophic substrates were used in the study. It had been established that iron ions at 6 h of incubation had a sharp increase of toxicity level of mixture; it was visualized with reduction of bacterial luminescence in comparison with the same system of 3 h incubation. Cupric copper ions showed much less toxic properties emerging only 6 h of incubation and demonstrating decrease of bacterial luminescence only by a few percents. The impact of physical factors by means of preliminary treatment by super high frequency waves or ultrasound formed a significant increase in toxicity during the first hours. Calcium cations had a positive effect on luminescence of recombinant *E. coli* strain. So, biotesting allows to determine biotoxicity of ruminal fluid with regard to feed components, degree of biosensors viability.

Key words: Cattle, evaluation, biotoxicity, ruminal fluid, biosensor

INTRODUCTION

The problem of emergence and prevention of toxicosis in ruminants is currently highly important including chronicity caused by heavy metals (Ashrafihelan *et al*., 2013), plant poisons (Foote *et al*., 2013). Some natural components cause sudden fatal toxic effect through the rumen of animals (Giannitti *et al*., 2013; Burcham *et al*., 2013). In addition, the active development of means for reduction of methane emissions into environment gives rise to the problem of their toxic effects on animals (Hristov *et al*., 2013; Drewnoski *et al*., 2014). After all, toxic effect causes the reduction of metabolism at the cellular level (Koontz *et al*., 2013). In this regard, the study of influence of feed components biotoxicity on ruminal microflora is a crucial task as especially this link of bacterial biocenosis firstly interacts with feed entering the rumen. One way to solve this problem; biotesting allowing to determine toxic level of environment according to the degree of biosensors viability (Kaiser and Esterby, 1991; Kaiser, 1998).

MATERIALS AND METHODS

Recombinant strain *E. coli* K12 TG1 with cloned luxCDABE-genes *Photobacterium leiognathi* 54D10 (Danilov *et al*., 2002), produced in form of lyophilized preparation with trade name «Ecolum-9» (Scientific Innovation Company «Immunotech», Moscow) and also series of reporter microorganism with inducible expression of lux-genes, cloned under different promoters were used in researches. The objects of the research were ruminal fluid of Kazakh white-headed beef cattle; model ruminal fluid obtained on the basis of phosphate buffer, propionic, lactic, butyric, acetic acid, glucose and 10% aqueous ammonia; trophic substrates based on wheat middling, extruded with Ca^{2+}, Cr^{2+}, Fe^{2+}, Cu^{2+} or treated with microwaves (MW) and ultrasound (US). In this case, 128 mL of ruminal fluid and 500 mg of trophic substrate were added in each container of "artificial rumen" and incubated for 24 h at 37°C. Time interval for sampling was 3, 6, 12, 24 h (Table 1).

Table 1: Scheme of artificial rumen

K_0	K	S+Ca	S+Cr	S+Fe	S+Cu	S+MW	S+US
Ko	K	S+Ca	S+Cr	S+Fe	S+Cu	S+MW	S+US
Ko	K	S+Ca	S+Cr	S+Fe	S+Cu	S+MW	S+US

K_0: Model ruminal fluid, K: Real ruminal fluid, S+Ca: Extruded wheat middling with addition of Ca^{2+}, S+Cr: Extruded wheat middling with addition of Cr^{2+}, S+Fe: Extruded wheat middling with addition of Fe^{2+}, S+Cu: Extruded wheat middling with addition of Cu^{2+}, S+MW: Wheat middling processed by MW, S+US: Wheat middling processed by US

After 24 h of incubation in the conditions of "artificial rumen" ruminal fluid was separated from substrate and from bacteria by centrifugation for 10 min and liquid was formed where it was determined if there was a toxicant in it. The level of bioluminescence of bacterial strains was expressed in units of bioluminescent index (BLI). Artificial rumen KPL-01 (Popov and Rybina, 1983), luminometer LM-01T (Immunotech, Czech Republic), pH meter ionomer Expert-001 (Eoniks-Expert, Russia), test-tube centrifuge CM-6M (Elmi, Russia) have been used as basic equipment for research.

RESULTS AND DISCUSSION

Research has focused on the study of the effect of feed factor on possible mechanisms of biological toxicity of ruminal fluid in conditions of artificial rumen. Thus, effects of various metal cations and physical methods of fodder processing with different intervals of exposure of ruminal fluid and feed factor were assessed.

First of all, the influence of incubation time of ruminal fluid in artificial rumen on the response character of the luminescent bacteria after 30 min exposure was estimated. It was found that toxicity level of ruminal fluid practically did not change and over time it was decreasing, it was manifested in a decrease of the inhibitory influence on bioluminescence of bacterial cells and even on increase of their luminescence level. However, the addition of elements in the form of divalent metals to trophic substrate as well as the use of additional physical processing methods significantly changed the picture of luminescent response of microorganisms. Thus, the iron ions after 6 h of incubation heavily increased the toxicity level of the mixture which was manifested in decreasing bacterial luminescence by 23.8% in comparison with the same system of 3 h incubation (Fig. 1).

Similar effects were associated with ability of divalent iron to start the formation of different reactive oxygen intermediates (Fenton reaction), where in the toxic effect consisted in interaction of the formed hydroxyl anions with structural and functional molecules of cells, including membrane phospholipids and nucleic acids. Use of a mixture containing iron, incubated within 12 h demonstrated a maximal effect of toxicity increase that reached 40.7% with respect to the mixture soaked for 3 h. On the other hand, the cupric ions demonstrated much less toxic properties, emerging only at 6 h of incubation and showing decrease of bacterial luminescence only for a few percent. Difference of

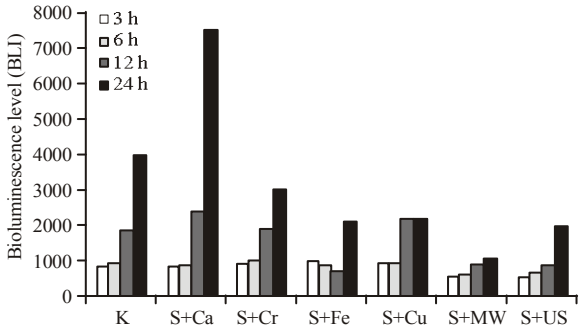

Fig. 1: Influence of incubation time and additional feed factors on level of integral toxicity of ruminal fluid

metal effects is determined by the fact that cupric copper is more stable ion being in its typical form of oxidation while ferrous iron is oxidized not in full and is able to send one more electron to acceptor that is present in the medium to form stable trivalent form. Earlier experiments revealed that the activity of the cellulase gene (Cel14b22) in *Escherichia coli* cells is sharply reduced due to Fe^{3+} or Cu^{2+} (Gong *et al*., 2012).

Another element demonstrating effects of strengthening the integrated toxicity of trophic substrate was chrome. However, the peculiarity of this ion was prolongation of developing effect, best of all expressed after a day of incubation in artificial rumen and account for 25.8% quenching of luminescence in comparison with the control samples with the same incubation time. Presumably the action of chromium was based on its strong reducing properties, leading to the accumulation of modified components of the substrate, having devastating effect on microorganisms.

The impact of physical factors in the form of pre-processing by microwaves or ultrasound has formed a significant increase in toxicity in the first hours while at the third hour luminescence quenching was characterized by values 52.7 and 51.6% in comparison with the control, respectively. Then due to the lack of subsequent impact of these factors on trophic substrate dynamics reflected the development of effects of the control samples, however, it was characterized by lower absolute values of bioluminescence of bacterial strains.

Only calcium cations had the positive effect on the luminescence of the recombinant *E. coli* strain, provided that trophic substrate supplemented with calcium ions intensified luminescence with increasing incubation time which in a day of exposure was 1.92 times greater than that obtained in the control samples. Similar results are likely connected with the fact that these cations reduced the level of acidity of the medium without the formation of toxic by-products as well as turned different components of denatured feedstock to the inactive forms.

CONCLUSION

Thus, effects of different metal cations and physical methods of feed processing with different intervals of exposure

of ruminal fluid with feed factor weer assessed. Adding elements in the form of divalent metals to the trophic substrate and also the use of additional physical processing methods significantly changed the picture of the luminescent response of microorganisms. So, the iron ions at 6 h of incubation drastically increased the level of toxicity of mixture which was manifested in decreasing bacterial luminescence by 23.8% in comparison with the same system of 3 h incubation. Cupric ions showed much less toxic properties, emerging only at 6 h of incubation and demonstrating decrease of bacterial luminescence only by a few percent. The impact of physical factors in the form of preliminary processing by microwaves or ultrasound has formed a significant increase in toxicity as early as the first hours. Only calcium cations had the positive effect on the luminescence of recombinant *E. coli* strain, provided that trophic substrate supplemented with calcium ions with increasing incubation time intensified luminescence which a day after exposure was 1.92 times greater than that obtained in the control samples.

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