

# EFFECT OF CORYNEBACTERIUM CUTIS LYSATE TREATMENT ON THE CYTOKINE LEVELS IN SHEEP



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## INTRODUCTION

Commercial product (Ultra-corn® Inj.) of *Corynebacterium cutis* lysate (CCL) stimulates nonspecific immune system in ruminants and poultry. Application of CCL increases the resistance to stress, viral, parasitic and bacterial infections and effects of vaccines (1). In addition, CCL increases Ig G levels of blood and colostrum; hence, it is thought to increase the survival rate of calves (2, 3). Similar data has been also reported in sheep (4).

In the infections, acute phase response (APR) proteins are synthesized. However, when APR is long time, adverse effects occur in the organism. Cytokines play important role in APR (5). The cytokines are defined as proinflammatory (tumor necrosis factor (TNF) (6), interleukin-1 (IL-1), IL-6) and anti-inflammatory (IL-10) (7). TNF is closely related to regulation of host immunity (8). Besides, there are many different effects of TNF in vivo, particularly as the main regulator of the proinflammatory cytokine response (9). IL-1 $\beta$  is mainly secreted by immune system cells (10). IL-6 plays a key role in the inflammation and allows secretion of factors such as serum amyloid-A with fibrinogen and C-reactive protein (11). The balance between inflammations and anti-inflammation has vital importance. IL-10, anti-inflammatory cytokine, is repressed to proinflammatory cytokines (TNF $\alpha$ , IL-1 $\beta$ , IL-6) and phagocytosis (7, 12). Measured some parameters (haemogram and biochemistry) from blood are retrieved information about the functionality of organs and some systems (13, 14). When CCL are phagocytosed by macrophages, macrophages stimulate the synthesis of cytokines (TNF $\alpha$ , IL-1 $\beta$ , IL-6) (15). However, there is no information in this regard about the CCL. In this research, it has been hypothesized that usage of CCL in order to immunostimulation in the different animals species may stimulate the synthesis of the cytokine in sheep as target species.

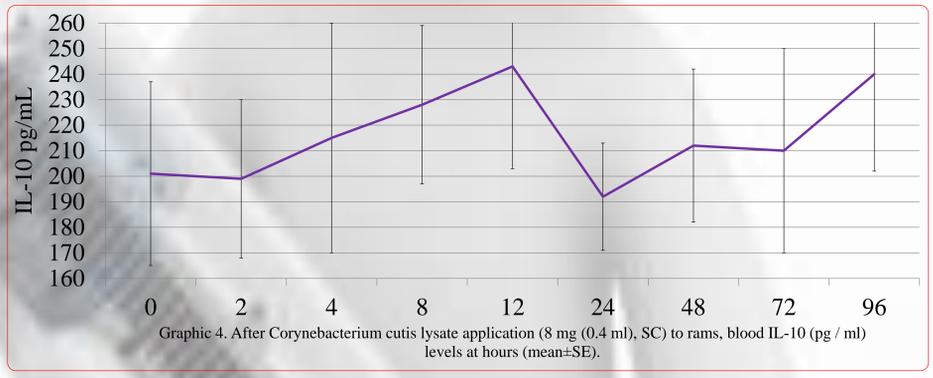
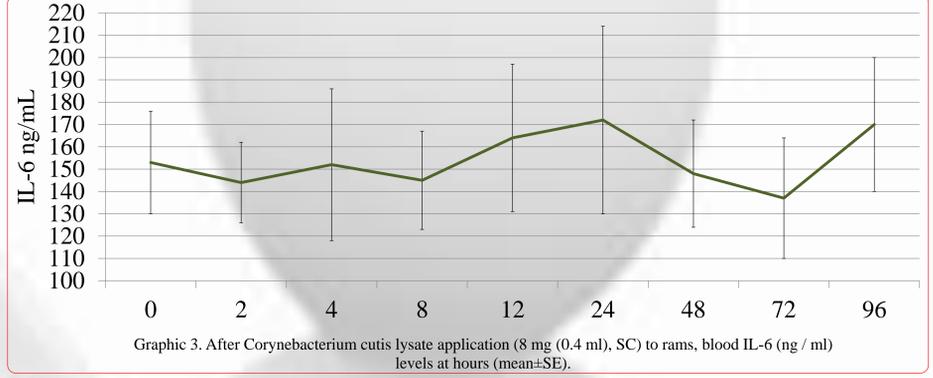
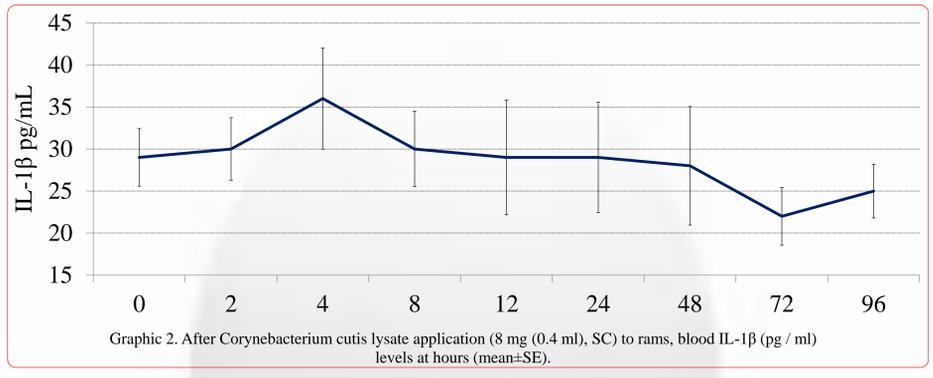
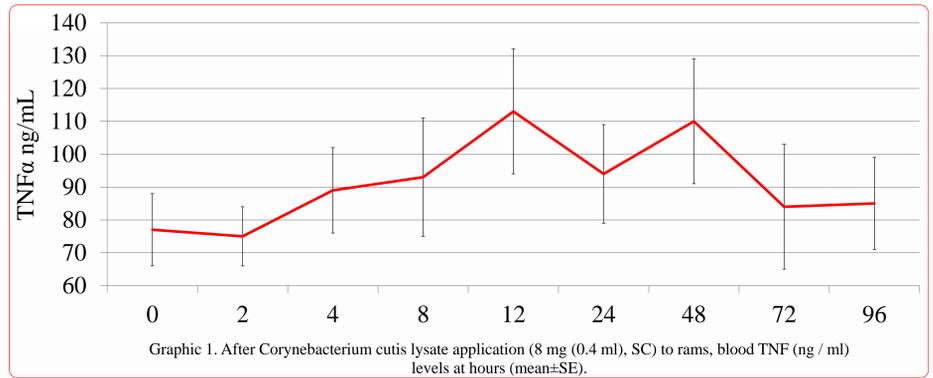
The aim of this research was to determine the effect of CCL on cytokine levels (TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-10) including hemogram and routine biochemical parameters in sheep.

## MATERIALS AND METHODS

In the research recommended dose (8 mg, 0.4 mL) of CCL (Ultra-corn® Inj.) was administered subcutaneously at a single dose to 10 male yearling Merino sheep. Before (0 hour, control) and after the treatments, blood samples were obtained from v. jugularis at 2, 4, 8, 12, 24, 48, 72 and 96 hours. Concentrations of serum Tumor necrosis factor alpha (TNF $\alpha$ ), Interleukin (IL)-1 $\beta$ , IL-6 and IL-10 were determined with ELISA reader. Hemogram values (white blood cell, red blood cell, platelet, hematocrit, hemoglobin) were determined with hemocell counter, whereas biochemical values (Creatine kinase-MB, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, total protein, albumin, blood urea nitrogen and creatinine) were analyzed with autoanalyzer. The data were analysed using ANOVA, and then Scheffe test as a post-hoc test. P value at less than 0.05 was considered statistically significant.

## RESULTS

CCL caused fluctuations on the cytokine levels ( $P>0.05$ ) (Graph. 1, 2, 3, 4). Hemogram parameters did not change statistically significant (Table 2), while fluctuations were determined ( $P<0.05$ ) in some biochemical values (Alkaline phosphatase, total protein, albumin, creatinine (Table 1).



Parameters	0. hour	2. hour	4. hour	8. hour	12. hour	24. hour	48. hour	72. hour	96. hour
CK-MB (U/L)	220 $\pm$ 24.9	232 $\pm$ 15.3	223 $\pm$ 11.3	248 $\pm$ 11.2	240 $\pm$ 14.6	244 $\pm$ 12.1	288 $\pm$ 11.5	282 $\pm$ 9.44	302 $\pm$ 12.8
ALP (U/L)	45.7 $\pm$ 4.90 <sup>a</sup>	50.1 $\pm$ 6.86 <sup>ab</sup>	50.0 $\pm$ 7.91 <sup>ab</sup>	48.5 $\pm$ 4.68 <sup>ab</sup>	78.0 $\pm$ 7.97 <sup>ab</sup>	83.7 $\pm$ 9.82 <sup>ab</sup>	81.7 $\pm$ 6.82 <sup>ab</sup>	90.3 $\pm$ 10.2 <sup>a</sup>	84.7 $\pm$ 5.35 <sup>ab</sup>
ALT (U/L)	14.6 $\pm$ 1.60	13.2 $\pm$ 1.37	13.7 $\pm$ 1.80	13.0 $\pm$ 1.59	12.9 $\pm$ 1.64	12.7 $\pm$ 1.17	14.4 $\pm$ 1.34	14.6 $\pm$ 1.40	13.0 $\pm$ 1.38
AST (U/L)	87.7 $\pm$ 6.73	80.4 $\pm$ 5.30	90.0 $\pm$ 13.3	107 $\pm$ 11.2	79.9 $\pm$ 5.61	83.6 $\pm$ 5.54	87.5 $\pm$ 4.16	81.6 $\pm$ 4.08	81.5 $\pm$ 4.35
GGT (U/L)	48.5 $\pm$ 3.19	45.5 $\pm$ 5.05	49.3 $\pm$ 4.98	61.6 $\pm$ 6.14	44.3 $\pm$ 2.85	46.1 $\pm$ 2.57	47.4 $\pm$ 2.82	48.9 $\pm$ 5.08	46.8 $\pm$ 3.40
Tpoc (g/dL)	5.22 $\pm$ 0.28 <sup>a</sup>	4.91 $\pm$ 0.46 <sup>a</sup>	5.09 $\pm$ 0.30 <sup>a</sup>	6.93 $\pm$ 0.46 <sup>b</sup>	7.34 $\pm$ 0.14 <sup>a</sup>	7.56 $\pm$ 0.13 <sup>a</sup>	7.87 $\pm$ 0.24 <sup>a</sup>	7.54 $\pm$ 0.17 <sup>a</sup>	7.51 $\pm$ 0.13 <sup>a</sup>
Alb (g/dL)	3.11 $\pm$ 0.05 <sup>ab</sup>	3.17 $\pm$ 0.15 <sup>ab</sup>	2.78 $\pm$ 0.18 <sup>b</sup>	3.75 $\pm$ 0.32 <sup>a</sup>	3.14 $\pm$ 0.07 <sup>ab</sup>	3.34 $\pm$ 0.06 <sup>ab</sup>	3.49 $\pm$ 0.08 <sup>ab</sup>	3.33 $\pm$ 0.60 <sup>ab</sup>	3.35 $\pm$ 0.05 <sup>ab</sup>
BUN mg/dL)	29.5 $\pm$ 2.50	33.3 $\pm$ 2.50	32.9 $\pm$ 4.18	25.1 $\pm$ 1.67	28.6 $\pm$ 2.13	30.2 $\pm$ 2.36	29.1 $\pm$ 1.75	36.5 $\pm$ 4.24	28.6 $\pm$ 0.91
Creatinine (mg/dL)	0.86 $\pm$ 0.04 <sup>ac</sup>	0.85 $\pm$ 0.06 <sup>a</sup>	0.90 $\pm$ 0.07 <sup>abc</sup>	1.36 $\pm$ 0.05 <sup>bc</sup>	1.21 $\pm$ 0.05 <sup>bc</sup>	1.37 $\pm$ 0.11 <sup>a</sup>	1.29 $\pm$ 0.06 <sup>bc</sup>	1.28 $\pm$ 0.08 <sup>bc</sup>	0.96 $\pm$ 0.15 <sup>bc</sup>

<sup>a,b,c</sup>: Different letters are statistically significant in terms of the same line ( $P<0.05$ ).

Parameters	0. hour	2. hour	4. hour	8. hour	12. hour	24. hour	48. hour	72. hour	96. hour
WBC ( $\times 10^9/L$ )	8.76 $\pm$ 0.40	9.69 $\pm$ 0.97	9.88 $\pm$ 1.05	10.1 $\pm$ 0.91	11.6 $\pm$ 1.07	9.91 $\pm$ 0.57	10.5 $\pm$ 0.77	10.2 $\pm$ 0.70	10.4 $\pm$ 0.91
RBC ( $\times 10^{12}/L$ )	10.5 $\pm$ 0.31	10.1 $\pm$ 0.29	10.4 $\pm$ 0.32	10.5 $\pm$ 0.31	10.2 $\pm$ 0.28	10.0 $\pm$ 0.32	10.6 $\pm$ 0.19	9.74 $\pm$ 0.31	9.86 $\pm$ 0.44
Platelet ( $\times 10^9/L$ )	241 $\pm$ 27.9	197 $\pm$ 20.6	218 $\pm$ 26.5	219 $\pm$ 25.7	228 $\pm$ 24.5	226 $\pm$ 13.4	235 $\pm$ 16.2	250 $\pm$ 48.0	219 $\pm$ 25.6
Hematocrit (%)	35.1 $\pm$ 0.87	33.7 $\pm$ 0.94	34.4 $\pm$ 0.73	34.8 $\pm$ 0.73	33.2 $\pm$ 0.81	33.1 $\pm$ 0.93	35.9 $\pm$ 0.62	32.0 $\pm$ 0.90	32.4 $\pm$ 1.21
Hemoglobin (g/dL)	8.87 $\pm$ 0.26	8.37 $\pm$ 0.27	8.68 $\pm$ 0.22	8.79 $\pm$ 0.24	8.38 $\pm$ 0.19	8.46 $\pm$ 0.28	9.07 $\pm$ 0.18	8.23 $\pm$ 0.22	8.21 $\pm$ 0.32

\* Statistical difference was not determined on the same line ( $p>0.05$ ).

## CONCLUSION

CCL is recommended for use as an immune system stimulant in ruminants (sheep, goats, calves, cattle). Also, it is reported that can improve vaccine efficacy when administered with vaccination (1). It is defined that when CCL is phagocytosed by macrophages (6), it can stimulate the synthesis of proinflammatory cytokines (TNF, IL-1 and IL-6) (14). In this research, TNF- $\alpha$  (Graph 1), IL-1 $\beta$  (Graph 2) and IL-6 (Graph 3) levels were detected fluctuations but not statistically significant ( $P>0.05$ ). Peak levels of TNF, IL-1 $\beta$  and IL-6 were determined at 12th, 4th and 24th hours, respectively. In the present study, CCL caused fluctuations in IL-10 (anti-inflammatory cytokine) level and it's peay concentrations was detected at 12th hour (Graph 4). IL-10 inhibits TNF, IL-1 and IL-6 synthesis in the body (7, 12). Although it has been reported that CCL may stimulate non-specific immune system in mastitis infection (4) and stimulate IgG synthesis in cows (3) and sheep (4), there has been no data found about the effect of CCL on the synthesis of cytokines. In addition, high antibody titers has determined when CCL administered with the vaccine in poultry (5) and cattle (6). It may be stated that treatment of CCL has no impressive effect on the cytokine levels, and detailed researches are need to determine the effect of CCL on the passive immunity. In this research, CCL had no important ( $P>0.05$ ) effect on the haemogram values (red blood cells, white blood cells, platelets, hemoglobin, hematocrit) (Table 2), whereas it caused fluctuations ( $P<0.05$ , Tale 2) in the some biochemical parameters (ALP, total protein, albumin, creatinine); however, this changes within reference range. It has been reported that CCL does not cause to oxidative stress, haemogram parameters and organs (heart, liver, kidney) damage on sheep (16, 17). As a result, it may be stated that CCL sold as an immune stimulatory commercially product has no impressive effect on the cytokine synthesis in sheep, however, to determine the effect of CCL on the immune system, more detailed molecular researches are need.

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