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The efficiency of propolis in post-colostral dairy calves

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Abstract

The study aimed at an assessment of a possibility of calves' health status improvement in neonatal period by preventive application of 10 % ethanol extract of propolis (EEP). An influence of EEP on selected biochemical and haematological parameters of blood, body weight gains and diarrhoea symptoms intensity was determined. Propolis contains a range of biologically active compounds and exhibits numerous beneficial properties. Ethanolic extract is a form of propolis that is usually used in prevention. Forty five calves were used in the experiment. The assessment of clinical symptoms of diarrhoea, dehydration and vitality was conducted and calves (without symptoms of diarrhoea) were divided into 3 groups (15 calves in each): control, and two experimental (2 and 4 ml of EEP/day). The results of the study point that EEP may be a useful mean improving health status of calves. After an application of propolis in a dose of 4 ml/day higher daily gains were noted when compared to the control calves. Although no obvious influence of EEP on haematological parameters was noted, the positive influence on erythropoiesis and Fe content was observed. Higher EEP dose caused a significant decrease in lactic acid (LA) level. No influence on macroelements and electrolytes in blood serum was noted.

Keywords: ethanol extract of propolis, calves, blood, health status

Introduction

The health of calves in neonatal period depends on numerous factors like passive immunity transfer, nutrition level, infection risk, maintenance conditions etc. Alimentary tract diseases manifested in a form of diarrhoea are the most common health problems in calves in the first weeks of their life. Etiology of diarrhoea is complex, since except environmental and nutritional factors, infectious factors are an important cause of that disease (García *et al.* 2000, Calloway *et al.* 2002, Sunderland *et al.* 2003). High percentage of calves' diseases incidents noted in various countries (García *et al.* 2000, Sunderland *et al.* 2003) is a reason of searching for new means aimed to improve their health status. Natural compounds are an alternative for chemotherapeutic agents (Ishihara *et al.* 2001), especially that the phenomenon of microorganisms resistance on antibiotics is more and more often mentioned in the professional literature (Gunn *et al.* 2003). Currently, when antibiotic growth stimulators (AGS) are not allowed any more for the common use, each new proposal seems to be an interesting one.

Propolis contains a range of biologically active compounds like phenol compounds, flavonoids (primuletin, chrysine, tecochrysine, akacetine, galangine, morin, robinetin), terpenes, lipid-wax substances, bioelements, vitamins (A, D, F, K, E, B_1, B_2, B_5, B_6, B_{12}, C, H, P), enzymes (alpha and beta amylase), amino acids, sterols, steroids, plant steroids, plant sterols (ergosterol, stigmasterol, steroidal saponins, steroidal alkaloids) (Marcucci 1995, Sahinler & Kaftanoglu 2005). Propolis exhibits antibacterial, antifungal, antiprotozoal, anaesthetic, regenerative, immunostimulating, anti-inflammatory, detoxic (forms chelate connections facilitating toxic compounds expelling), antioxidative properties (Borelli *et al.* 2002, Sforcin 2007). Ethanolic extract is a form of propolis that is used the most often in prevention. In the study on broiler chickens subject to thermal stress, the profitable effect of ethanol extract of propolis (EEP) on antioxidative enzymes level and level of some blood parameters was observed (Seven *et al.* 2009). The additional propolis advantage as a preventive preparation is the fact, that no microbes' resistance on its activity was observed.

Calves of Simmental breed have not been the subject of the study concerning an application of propolis in post-colostral dairy calves feeding. Thus, the aim of the study was an assessment of a possibility of an improvement of health status of calves in neonatal period by preventive application of ethanol extract of propolis (EEP). An influence of EEP on selected biochemical and haematological parameters of calves blood, body weight gains and diarrhoea symptoms intensity was determined.

Material and methods

The study was conducted on calves of Simmental breed in dairy type in winter-spring period. The animals were kept on a farm of 250 cows in Czech Republic. Calves were kept in a nursery (group pens), and up to the 3rd day of life they were given colostrum. At the 3rd day of life they were moved to individual pens. From the 3rd day of life they were given whole milk, and from the 6th day milk replacer (Sanolac Rot, Sano, Sękowo,Poland) and a complete feed mixture of starter type (Maggi, Sano, Sękowo, Poland). They had *ad libitum* access to water.

Forty five calves were chosen for the experiment on the analogue basis, taking into consideration age, body weight and gender. The assessment of clinical symptoms of diarrhoea, dehydration and vitality was conducted at 7th day of life according to point scale from 0 to 3 (Table 1). Animals were divided into 3 groups (15 calves in each):

- group I – control, that was not given the preparation, without symptoms of diarrhoea,
- group II – without symptoms of diarrhoea, application of ethanol extract of propolis (EEP) from 7th to 14th day of life in amount of 2 ml/day,
- group III – without symptoms of diarrhoea, application of EEP from 7th to 14th day of life in amount of 4 ml/day,

In the case of groups II and III, the 10 % ethanol extract of propolis was used (preparation standardised by the manufacturer – 100 mg of propolis/ml). The preparation was given to calves once a day together with milk replacement.

Blood from an external jugular vein was collected from all the calves at 7th, 14th and 21st day of their life, before feeding. The determination of biochemical parameters of blood was done using ABX Pentra 400 biochemical analyser (Horiba ABX SAS, Montpellier, France), while

of haematological parameters using Sysmex K-4500 haematological analyser (Sysmek, Kobe, Japan). The following analyses were conducted:

- haematological parameters: haematocrit (HCT), haemoglobin (HGB) concentration, white blood cell count (WBC) and red blood cell count (RBC); red blood cell indices, i.e. mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated.
- biochemical parameters of blood serum: glucose by oxidase method with use of Horiba ABX SAS reagents (Glucose PAP, A11A01668), lactic acid (LA) using oxidase/peroxidase method (LA, A11A01721), total protein and albumins using colorimetric method with Horiba ABX SAS reagents (Total Protein CP, A11A01669; Albumins CP, 11A01664).
- activity of enzymes: aspartate aminotransferase (AST) and γ- glutamylotransferase (GGT), lactic dehydrogenase (LDH) by kinetic method according to IFCC recommendations using Horiba ABX SAS reagents (AST CP, A11A01629; GGT CP, A11A01630; LDH CP, A11A01824).
- Ca concentration using photometric method with ortho-cresoftalein complex (Ca CP, A11A01633), inorganic P using UV method with phosphomolybdate (P CP, A11A01665), Mg using photometric method with xylidyl blue (Mg RTU, A11A01646), Fe using photometric method with pherene (Fe CP, A11A01637), Na, K and Cl using ISE, with the three ionoselective electrodes with separate membranes.

Table 1
An assessment of clinical symptoms of diarrhoea, dehydration and vitality of calves (according to Sunderland *et al.* [2003] in an own modification)

Clinical symptoms	Occurrence/intensity of clinical symptoms			
	Absent 0 pts	Mild 1 pts	Moderate 2 pts	Acute 3 pts
Diarrhoea	Normal excreta	Loose or »muddy«, but formed excreta.	Loose or watery, unformed excreta.	Abundant, watery excreta, presence of mucus, blood.
Dehydration	Directly after stretching, the skin comes back to a normal position.	The skin comes back to a normal position after a short time (<3 s).	Moderate skin tension, comes back after 4-7 s.	The skin comes back to a normal position after more than 7 s.
Overall condition	Vitality, vigilance, proper reaction on stimuli.	Lowered reaction on stimuli. Weakened appetite.	Lowered. May unwillingly keep a standing position.	Critical. Unable to stay without a help.

The calves were weighted at 7th and 21st day of life and daily body weight gains in that period were determined on that basis.

Basic statistical parameters of results (means, standard deviations) in individual groups were determined using the Microsoft Excel XP software (Microsoft, Redmond, WA, USA). The significance of differences in average values in relation to several monitored periods between the groups was evaluated by one way analysis of variance ANOVA (Statistica 8.0, StatSoft, Inc. Tulsa, OK, USA). The significance of differences between the experimental factors was determined by Duncan's test. $P \leq 0.05$ and $P \leq 0.01$ were considered to be statistically significant.

Results

Body weight of calves in particular research periods and daily gains did not differ between the particular groups (Table 2). The highest body weight at 21st day of life was noted in calves that were given 4 ml/day of propolis (48.4 kg). The lack of daily gains in group I may be explained by the fact that in several calves moderate diarrhoea symptoms were noted at 14th day of life. As a result, these calves consumed smaller amount of starter feed.

Table 2
Body weight and daily gains of calves (mean±standard deviation)

Specification	Body weight/days of life			Daily gains, g
	7th	14th	21st	
Control	44.5±7.86	45.9±7.82	44.6±7.59^{a}	7.1
Propolis 2 ml/day	45.6±5.95	47.1±6.39	46.7±5.49	79
Propolis 4 ml/day	45.3±5.87	47.1±5.39	48.4±4.35^{b}	221

^{a,b}Differences between the groups are significant statistically on the level of $P<0.05$.

The results of clinical examination are presented in Table 3. The highest diarrhoea intensity was noted at the 7th day of life in calves from experimental groups. An application of the preparation in amount of 4ml/day caused a significant ($P\leq0.01$) decrease in a point-scale assessment of diarrhoea intensity. At the 14th and 21st day of life, an overall condition of calves and also dehydration assessment, were more advantageous in calves that were given both doses of the preparation. No typical, acute clinical symptoms of alimentary tract disorders were observed during the whole research period.

Table 3
An assessment of clinical symptoms of diarrhoea, dehydration and vitality of calves (mean±standard deviation)

Group	Diarrhoea	Dehydration	Overall condition
7th day of life			
Control	0.25±0.27^{a}	0.38±0.24$^{\underline{A}}$	0.44±0.30
Propolis 2 ml/day	0.44±0.27^{b}	0.43±0.35	0.43±0.23$^{\underline{a}}$
Propolis 4 ml/day	0.44±0.24$^{b\underline{A}}$	0.44±0.38$^{\underline{a}}$	0.46±0.28$^{\underline{a}}$
14th day of life			
Control	0.63±0.24^{A}	0.75±0.46$^{A\underline{B}}$	0.44±0.68^{A}
Propolis 2 ml/day	0.41±0.32	0.59±0.37	0.35±0.38
Propolis 4 ml/day	0.25±0.46$^{B\underline{B}}$	0.26±0.18$^{B\underline{b}}$	0.23±0.23$^{B\underline{b}}$
21st day of life			
Control	0.48±0.22^{A}	0.56±0.68^{A}	0.38±0.22^{a}
Propolis 2 ml/day	0.50±0.41	0.36±0.24	0.21±0.27$^{\underline{b}}$
Propolis 4 ml/day	0.19±0.24$^{B\underline{B}}$	0.17±0.19$^{B\underline{b}}$	0.23±0.18$^{b\underline{b}}$

^{a,b}differences between the groups at a given day significant on the level of $P\leq0.05$, ^{A,B}differences between the groups at a given day significant on the level of $P\leq0.01$, $^{\underline{a},\underline{b}}$differences between the periods (days) within the group significant on the level of $P<0.05$, $^{\underline{A},\underline{B}}$differences between the periods (days) within the group significant on the level of $P<0.01$

The values of biochemical parameters of calves blood and AST and GGT activity are presented in Table 4. At the day of the beginning of the experiment, no statistical differences in a range of analysed parameters, except LA, were noted between the groups. Glucose concentration in blood serum was subject to a significant decrease ($P\leq0.01$) up to the 21st day of life in all

the groups, in the control group the decrease was from 5.33 to 4.90 mmol/l, while the highest was in the group that was given 4 ml/day of propolis (from 5.65 to 4.22 mmol/l). At the day of the beginning of the experiment, higher LA concentration ($P \leq 0.05$) was observed in group III (2.02 mmol/l) when compared to group I (control - 1.67 mmol/l). Higher LA concentration was probably the effect of mild diarrhoea symptoms resulting from the change in feeding. An application of 4 ml/day EEP caused a decrease ($P \leq 0.01$) in LA level in subsequent blood analysis (up to 1.38 mmol/l at 21st day of the study). The total protein (TP) level was subject to a systematic decrease in all the groups during the research period. That decrease was significant ($P \leq 0.01$) up to the 14th day of life in the case of calves from the control group. The changes in LA and TP concentration observed in the present study are not fully reflected in point assessment of diarrhoea intensity and organism dehydration degree (Tables 3 and 4). AST activity was subject to a significant decrease ($P \leq 0.01$) in calves from groups I and II up to the 14th day of their life (from 32.13 to 28.98 U/l and from 40.76 to 31.83 U/l, respectively). In the case of group I, AST activity increased ($P \leq 0.01$) to 38.75 U/l at the 21st day of calves life (Table 4). Similar increase ($P \leq 0.01$), however from the 7th to 21st day of life, was observed in calves from group III (from 28.32 to 39.77 U/l). Despite some changes, no differences in GGT activity were noted in particular groups (Table 4).

Table 4
Metabolites and enzymes activity concentration in serum of dairy cows (mean±standard deviation)

Parameters	Day	Group		
		Control	Propolis 2 ml/d	Propolis 4 ml/d
Glucose, mmol/l	7	5.33±1.02^{A}	5.70±0.8^{A}	5.65±0.66^{A}
	14	4.26±0.55^{B}	4.46±0.67^{B}	4.22±0.48^{B}
	21	4.90±0.93^{B}	4.64±0.86^{B}	4.22±0.66^{B}
LA, mmol/l	7	1.67±0.4^{a}	1.91±0.62	2.02±0.81$^{A\underline{b}}$
	14	1.55±0.63	1.64±0.69	1.28±0.3^{B}
	21	1.30±0.26	1.37±0.32	1.38±0.59^{B}
Total protein, g/l	7	55.92±12.82^{A}	59.49±5.48^{a}	55.98±8.92^{a}
	14	51.78±8.45^{B}	54.56±4.61^{b}	53.39±6.93^{b}
	21	54.08±7.17	56.87±5.6	54.94±6.84
Albumins g/l	7	23.22±1.7	24.11±1.43	24.29±1.84
	14	23.88±1.82	24.69±1.46	25.18±1.32
	21	24.92±1.98	26.01±1.46	26.65±1.07
AST, U/l	7	32.13±8.36^{A}	40.76±14.23^{A}	28.32±4.29^{A}
	14	28.98±6.53^{A}	31.82±5.48^{B}	31.58±5.11^{A}
	21	38.75±12.83^{B}	39.63±13.9^{A}	39.77±11.76^{B}
GGT, U/l	7	15.52±9.17	19.80±2.36	18.93±2.61
	14	20.32±8.81	17.41±6.48	20.54±8.9
	21	16.93±6.49	19.51±4.09	21.93±4.23

^{a,b}differences between the days of blood collection within the group significant on the level of $P<0.05$, ^{A,B}differences between the days of blood collection within the group significant on the level of $P<0.01$, ^{a,b}differences between the groups significant on the level of $P<0.05$

Table 5
The content of macro and microelements in blood serum of calves (mean±standard deviation)

Parameters	Day	Group		
		Control	Propolis 2 ml/d	Propolis 4 ml/d
Ca, mmol/l	7	2.37±0.35^{A}	2.40±0.28^{A}	2.31±0.38^{A}
	14	1.98±0.17^{B}	2.01±0.19^{B}	2.00±0.19^{aB}
	21	1.96±0.12^{B}	2.03±0.14^{B}	2.22±0.26^{b}
P, mmol/l	7	2.28±0.19	2.26±0.21	2.28±0.28
	14	2.18±0.19	2.21±0.18	2.31±0.16
	21	2.28±0.23	2.28±0.28	2.29±0.21
Mg, mmol/l	7	0.80±0.12	0.81±0.10	0.84±0.1
	14	0.75±0.09	0.78±0.09	0.81±0.1
	21	0.81±0.07	0.81±0.08	0.79±0.08
Na, mmol/l	7	134.49±3.54	136.49±2.47	135.56±1.54
	14	135.24±2.38	135.24±2.34	134.83±1.83
	21	136.24±2.9	136.56±3.23	135.16±1.54
K, mmol/l	7	5.28±0.46	5.32±0.44	5.12±0.34
	14	5.25±0.33	5.38±0.35	5.44±0.33
	21	5.32±0.42	5.23±0.47	5.28±0.32
Cl, mmol/l	7	100.42±2.03	102.05±2.51	99.21±2.5^{a}
	14	102.15±2.2	103.74±2.41	103.34±2.28^{b}
	21	102.17±1.63	104.11±2.59	102.42±2.26
Fe, µmol/l	7	12.2±5.29^{aA}	11.69±7.57^{A}	11.82±7.67^{A}
	14	14.41±4.56^{b}	17.94±12.23^{B}	16.65±7.82^{B}
	21	17.11±6.52^{B}	18.17±8.77^{B}	20.14±8.59^{B}

^{a,b}differences between the days of blood collection within the group significant on the level of $P<0.05$, ^{A,B}differences between the days of blood collection within the group significant on the level of $P<0.01$

Table 6
The haematological parameters in blood of calves (mean±standard deviation)

Parameters	Day	Group		
		Control	Propolis 2 ml/day	Propolis 4 ml/day
WBC, g/l	7	7.8±1.8	9.3±1.8	7.9±1.9
	14	8.2±2.1	8.6±2.1	7.4±1.6
	21	8.6±2.4	9.4±3.9	8.3±3.4
RBC, T/l	7	6.8±1.24	7.19±1.2	6.65±1.05^{a}
	14	7.02±2.15	7.44±1.2	7.05±1.01^{b}
	21	6.83±1.49	7.34±1.12	7.11±0.99
HGB, mmol/l	7	7.39±22.5	7.65±19.2	7.16±17.8^{a}
	14	7.76±21.1	8.17±18.6	7.68±16.3
	21	7.91±2.6	8.34±19.6	8.03±16.6^{b}
HCT, l/l	7	0.32±0.06	0.33±0.11	0.31±0.08
	14	0.33±0.05	0.34±0.10	0.33±0.05
	21	0.32±0.08	0.34±0.06	0.34±0.06
MCV, fL	7	46.3±0.9	46.1±1.1	46.1±1.1
	14	46.2±1.1	46.0±1.0	46.4±0.9
	21	46.4±0.9	46.5±1.3	46.4±0.8
MCH, pg	7	17.5±0.7	17.2±0.5	17.4±0.5
	14	17.8±0.7	17.8±0.6	17.6±0.6
	21	18.7±0.9	18.3±0.8	18.2±0.6
MCHC	7	23.09±0.79	23.18±1.41	23.10±2.17
	14	23.51±1.23	24.03±1.94	23.27±1.90
	21	24.72±1.88	24.53±1.29	23.62±1.44

^{a,b}differences between the days of blood collection within the group significant on the level of $P<0.05$

No statistical differences in an organic P content, Mg, Na and K in particular groups and blood samplings were observed. Ca concentration in blood serum was subject to a decrease ($P \leq 0.01$) up to 14th day of life in all the groups (Table 5). In the case of groups I and II, it was on a similar level at the 21st day of life (1.96 and 2.03 mmol/l, respectively), while increased in group III ($P \leq 0.05$) up to 2.22 mmol/l). The concentration of Fe increased gradually ($P \leq 0.01$) in all the groups during the period of the study (Table 5). A distinct increase ($P \leq 0.01$) in Fe concentration was noted after one week of EEP application (groups II and III). The highest Fe level at the 21st day of life was observed in group II (20.14 mmol/l).

Mean values of haematological parameters are presented in Table 6. In the case of calves that were given the propolis in amount of 4 ml/day, the erythrocytes number increased ($P \leq 0.01$) from 6.65 T/l to 7.05 T/l at the 14th day of life. Some increase in haemoglobin concentration was noted in the case of both experimental groups. The highest HGB concentration was found at 14th and 21st day of life in calves from group II (8.17 and 8.34 mmol/l, respectively). Red blood cell indices were not related to the preparation applied.

Discussion

For many years, there has been a strong interest in propolis as a therapeutic substance, not only in the case of human, but in a prevention and treatment of animals as well (Onlen *et al.* 2007, Seven *et al.* 2009). It was demonstrated that flavonoids extracted from propolis affect the humoral immune response and can improve growth in young calves (Yaghoubi *et al.* 2008). Depending on the geographical region and climatic conditions, the propolis is characterised by a specified chemical composition and biological properties (Kujumgiev *et al.* 1999).

The body weight gains observed in the present study were relatively low. The lack of body gains was observed in control animals, while in calves receiving 4 ml EEP/day they were the highest. Low body weight gains in the case of control animals resulted probably from the short-term diarrhoea observed in single individuals. After an application of flavonoids extracted from propolis higher body weight was noted when compared to those fed no or low doses of flavonoids (Yaghoubi *et al.* 2008). Lack of influence on production effects of beef calves was noted after an application of 0.05 % of propolis given with concentrate, what would have resulted from its low dose (Sarker & Yang 2010).

The concentration of glucose at the beginning of the experiment noted in the present study was similar to values given by Knowles *et al.* (2000). The decrease in glucose concentration up to 21st day of life was not related to the preparation given. An application of 4 ml/g of EEP caused a decrease ($P \leq 0.01$) in LA concentration from 2.02 to 1.28 mmol/l. In the case of healthy calves, the concentration of DL-lactic acid was 1.7 mmol/l, while 8.9 mmol/l was observed in the case of animals with diarrhoea (Omole *et al.* 2001). The changes observed in the present study should be considered as beneficial ones. In the present study, after 14 days of an application of higher EEP dose, the profitable influence on calves condition and diarrhoea symptoms occurrence was observed, especially when compared to the control calves (Table 3). Yaghoubi *et al.* (2007) have recently demonstrated that propolis flavonoids can act against protozoa and gram positive bacteria.

In the case of chronic dyspepsia of calves, when the metabolic acidosis occurs, the increase of GGT and K and Cl concentration was observed (Stocker *et al.* 1999). The increase

in Cl concentration observed in the present study in calves that were given propolis, was in the middle of the range of physiological values (Meyer & Harvey 1998). In the case of calves with diarrhoea, the concentration of Na and Cl was higher as compared to the present study (Omole *et al.* 2001). The tendency of changes in total protein and albumins concentration observed in the present study was connected to an age of animals. Knowles *et al.* (2000) observed a decrease in total protein level and a gradual increase in albumins level from 7th to 30th day of calves' life.

Both EEP doses did not demonstrate any clear influence on macroelements and electrolytes content in blood serum. The level of Ca observed in the present study was comparable to that reported by Matusevičius *et al.* (2008) in blood serum of healthy cows, or by another authors (Dvořák *et al.* 1980, Otto *et al.* 2000, Kupczyński & Chudoba-Drozdowska 2002). Mg concentration observed in the present study was also similar in all the groups and days of sampling. It was on a level comparable to that found in the literature (Dvořák *et al.* 1980, Matusevičius *et al.* 2008) or a bit lower (Shrikhande *et al.* 2008). Similar tendency was noted in the case of phosphorus content. The one observed in the present study was generally comparable to another author's observations (Dvořák *et al.* 1980) or lower (Kupczyński & Chudoba-Drozdowska 2002, Matusevičius *et al.* 2008). Only Shrikhande *et al.* (2008) noted higher level of phosphorus in blood serum.

At the day of the beginning of the present study, the level of Fe in blood serum was relatively low. In another study, the high differentiation in Fe content in blood serum, i.e. from 10.39 mmol/l to 20.33 mmol/l was noted at 7th day of life (Kupczyński *et al.* 2008). Mohri *et al.* (2007) observed the level of Fe in blood serum below 15 mmol/l 1-2 day after calving, and its increase up to 20 mmol/l around 2nd month of life. In the case of one-week old calves, the observed Fe content was 19.22 mmol/l (Mohri *et al.* 2004). High content of Fe in milk replacement and an intake of nutritive fodders by calves enabled a quick adjustment of slight iron balance disorders. In the third week of calves' life, the value was as high as 22.75 mmol/l (an initial value was 10.39 mmol/l) (Kupczyński *et al.* 2008). In the case of anaemia that takes place in chronic diseases, the proper concentration of Fe and lowered of TBC, and proper or increased ferritin concentration is observed (Jones & Allison 2007). The highest Fe level at the end of the present study was noted in calves that were given 4 ml/day of propolis, with a concurrent HGB increase ($P \leq 0.05$). The tendency of an increase in red blood cell count was noted at the same time (group III).

No influence of preparations applied on the values of particular haematological parameters was observed in the present study. In spite of some fluctuations, all the results obtained were within the range of reference values (Jones & Allison 2007). In the case of calves with diarrhoea symptoms, an increase in haematocrit level, and also HGB and MCHC was noted, that was accompanied by a decrease in MCV level (Constable *et al.* 2001). Such changes were not observed in the present study.

The results of the study conducted point that EEP may be a useful mean improving health status of calves. After an application of 4 ml/day of propolis, higher daily gains were noted when compared to control calves. The differences were not confirmed statistically. Although no obvious influence of EEP on haematological parameters was noted, the positive influence on erythropoiesis and Fe content was observed. Higher EEP dose caused a decrease in LA level. No influence on macroelements and electrolytes in blood serum was noted. The use

of a higher dose could probably allow reducing the time of application of the preparation necessary to cause apparent effects on calves.

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