THE EFFECT OF SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*) EXTRACT ON *CRYPTOSPORIDIUM* SPP. INVASION IN CALVES

Alīna Derbakova, **Dace Keidāne**, **Laima Liepa**, **Evita Zolnere**
Latvia University of Agriculture
alina.derbakova@gmail.com

Abstract
The present work aimed to evaluate the effect of sea buckthorn (*Hippophae rhamnoides*) extract on *Cryptosporidium* spp. invasion in calves. Sea buckthorn is a good source of vitamins, carotenoids, organic acids and tannins. It has been reported that it possesses anti-inflammatory effect, as well as anthelmintic and antibacterial activity. Research was conducted in a dairy cattle farm 'Mežacīruļi' during April and July 2015. Experimental (n = 10) and control (n = 10) groups of calves were used. The experimental group received a mix of aqueous alcohol sea buckthorn leaf and berry pomace extract, administered with milk, at dose of 5 – 8 mL twice a day for 20 days. The control group received only milk. Samples were analysed with the concentration McMaster technique. The results showed that there is no significant ($p>0.05$) difference in the number of oocysts per gram of feces between the experimental and control groups. It was concluded that the administration of sea buckthorn leaf and berry pomace extract had no effect on *Cryptosporidium* spp. invasion in calves. Studies will be continued with a higher dose of extract.
Key words: sea buckthorn, tannins, Cryptosporidium, calves, Latvia.

Introduction

Cryptosporidiosis is recognized worldwide, primarily in neonatal calves but also in lambs, kids, foals, and piglets. There are currently 19 species and 40 genotypes of *Cryptosporidium* spp. *C. parvum* is a common cause of calf diarrhea (mild to moderate), and cryptosporidium oocysts have been detected in the feces of 70% of 1- to 3-week-old dairy calves. Calves with persistent diarrhea have villous atrophy in the small intestine (O'Donoghue, 1995). Infection can be detected as early as 5 days of age, with the greatest proportion of calves excreting organisms between days 9 and 14 (Current, 1985).

The berries of sea buckthorn have been reported to be a good source of vitamins (A, C, E and K), carotenoids, flavonoids and organic acids (Gutzeit *et al*., 2008). Epigallocatechin and ursolic acid isolated from the branches of sea buckthorn exhibited anti-inflammatory effects (Kallio, Yang, & Peippo, 2002). Sea buckthorn fruits produced in Latvia are used as additives in acidified milk products (Segliņa, 2007). The shoots and green berries of female sea buckthorn plant contained the highest concentrations of condensed tannins, reaching 22.47 and 23.29 mg g^{-1} dry weight, respectively, which was about 20 times higher than in leaves of both genders (Šnē *et al*., 2013). However, it is mentioned, that the level of tannins in sea buckthorn can reach 13 mg g^{-1} condensed tannins (Michel *et al*., 2012).

Tannins are widely distributed in the plant kingdom (Mole, 1993). The ability of tannins to bind with proteins in the guts of mammals can have beneficial effects, depending on tannin concentration and nutrient levels. In cattle and other ruminants, moderate concentrations of condensed tannins (2 – 4% dry weight) reduce foaming of protein rich forage in the rumen and improve amino acid availability (McSweeney *et al*., 2001). Some tannin-rich forage has been evaluated for its anthelmintic and antibacterial activity (Mueller-Harvey, 2006). Tannins provided anthelmintic activity in many *in vitro* tests, adult nematodes exposed to commercial tannin extracts, from 25 mg mL^{-1} to 100 mg mL^{-1}, showed no motility inhibition after different exposure times. The authors suggested that adult worms may be more resilient to tannins compared to the larval stages (Iqbal *et al*., 2007).

There are no studies about anthelmintic effect of sea buckthorn on ruminants, but it is reported to possess unique health benefits. The main aim of the present study was to evaluate the effect of a mix of aqueous alcohol sea buckthorn *Hippophae rhamnoides* leaf and berry pomace extract on *Cryptosporidium* spp. invasion in calves.

Materials and Methods

Between April and July 2015, we collected fresh fecal specimens from newborn calves in the dairy cattle farm 'Mežacīruļi'. Animals were divided into two groups (n = 10 in each group). Starting with the second feeding the experimental group orally received a mix of aqueous alcohol sea buckthorn (*Hippophae rhamnoides*) leaf and berry pomace extract, which contains 3.2 g tannins in 100 mL. The starting dose was 5 – 8 ml, administered with milk, twice a day for 20 days. The control group received only milk. No other food supplements, drugs or vitamin injections were given or made. Rectal fecal samples were collected separately from each calf every day for 31 days, collected into plastic bags and kept in a refrigerator at 4 °C prior to examination. If the amount of feces was too small (especially in the first days of calves' life), native smears were made.

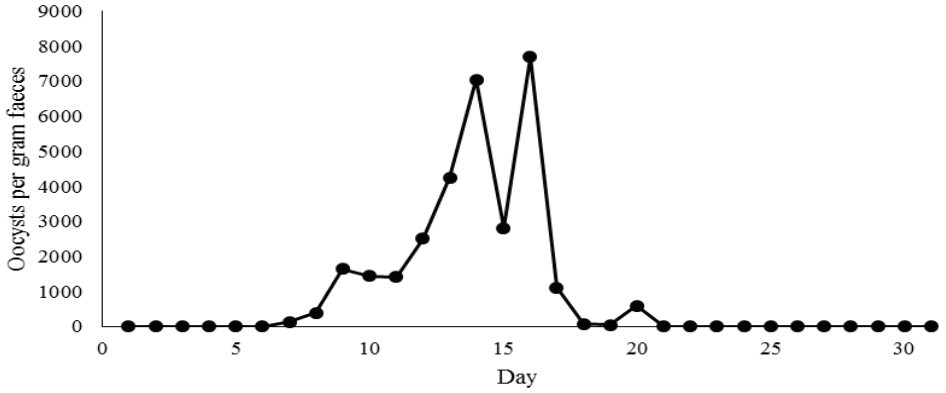

Figure 1. Shedding of oocysts in experimental group.

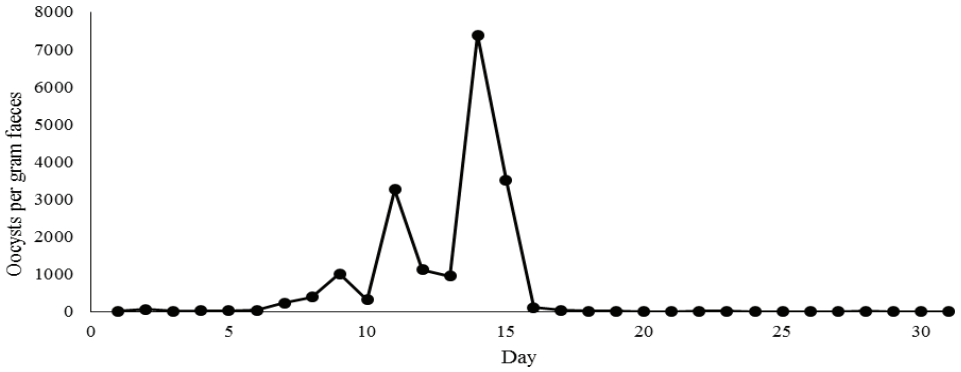

Figure 2. Shedding of oocysts in control group.

Laboratory tests were made in the Laboratory of parasitology, Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture. Samples were analyzed both with the concentration McMaster technique (Novan *et al*., 2006) and modified Ziehl-Neelsen method (Henriksen & Pohlenz, 1981). The difference in number of oocysts in the experiment and control groups was calculated using the t-test function in Microsoft Excel 2013 program.

Results and Discussion

Our research showed that calves in both groups were positive for oocysts of *Cryptosporidium* spp.

The main difference between two groups was in the number of bacteria in the stains. No identification of the bacterium was made. There were no bacteria in samples from the experimental group and a lot of bacteria in samples from control group during the first eleven days. After the eleventh day, no bacteria were matched. It could be explained with antibacterial activity of tannins (Funatogawa *et al*., 2004).

Shedding of oocysts in the experimental group is shown in Figure 1.

In the experimental group, the first *Cryptosporidium* spp. oocysts were detected in the feces starting with the seventh day. The highest oocysts count per gram of feces was on the fourteenth and sixteenth day (7050 and 7700 oocysts, respectively). After the twentieth day the number of oocysts was sharply reduced - from 585 oocysts on the twentieth day to 2.5 oocysts on the twenty first day. There are few reports on effects of tannins on new-born calves' digestive system. *In vitro* experiments (Khiaosa-Ard *et al*., 2009; Vasta *et al*., 2009) show positive effects on rumen, *in vivo* studies seem to suggest no significant or even negative effects (Benchaar & Chouinard, 2009).

Shedding of oocysts in the control group is shown in Figure 2.

In samples from the control group *Cryptosporidium* spp. oocysts were found starting with the first day. At the same time the number of oocysts per gram of feces on the fourteenth day was almost the same as in the experimental group – 7385 and 7050 oocysts, respectively. After the sixteenth day shedding of oocysts decrea sed and completely stopped after the nineteenth day. These findings are similar to the previous studies which report that *Cryptosporidium*

spp. infection in calves occurs at 5 – 15 days of age (Constable, 2014).

Our research showed that the administering of sea buckthorn leaf and berry pomace extract, containing 3.2 g tannins in 100 mL did not change *Cryptosporidium* spp. invasion ($p>0.05$). Therefore, we will continue studies to determine the effect of a higher dose of sea buckthorn extract on *Cryptosporidium* spp. invasion.

Conclusion

1. In the experimental group first *Cryptosporidium* spp. oocysts were detected in the feces starting with the seventh day. In the control group *Cryptosporidium* spp. oocysts were found in fecal samples starting with the first day.
2. The highest number of oocysts per gram of feces in both groups was on the fourteenth and sixteenth day in the experimental group and on the fourteenth day in the control group.
3. The administering of sea buckthorn leaf and berry pomace extract, containing 3.2 g tannins in 100 mL, did not change *Cryptosporidium* spp. invasion in calves ($p>0.05$). Studies will be continued with a higher dose of extract.

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