

***In vivo* Anticoccidial Activity of *Yucca schidigera* Saponins in Naturally Infected Calves**

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Abstract: A 75 day study was conducted comparing the anticoccidial efficacy of monensin and *Yucca schidigera* saponins (YS) in calves with naturally infection. A total 27 beef cattle selected for body weight and degree of oocysts shedding were allocated to three groups of 9 animals each: MON (Monensin, 140 mg/animal/day), YS (*Yucca schidigera* saponins, 15 g/animal/day) and CTRL (non-treated control). Individual faecal samples were collected at day 0, 15, 30, 45 and 75 to evaluate oocysts excretions (OPG), faecal consistency score and dry faecal percentage; body weight was recorded at day 0 and 75. On day 15, OPG were significantly lower in YS and MON compared to CTRL ($p = 0.014$ and 0.017 , respectively). From day 30 to the end of the study, OPG values were similar in all groups and a complete coccidial elimination was not recorded in any group. Faecal scores and dry-faecal percentages did not differ significantly between groups throughout the study. The highest mean daily weight gain was recorded for MON (1.73 kg/hd/day) with respect to YS (1.45 kg/hd/day) and CTRL (1.32 kg/hd/day). This study suggests that *Yucca schidigera* saponins under conditions of natural exposure to coccidiosis and normal management practices have anticoccidial activity and that a little advantage in gain can be obtained.

Key words: *Eimeria* sp., *Yucca schidigera*, saponins, beef cattle, coccidiosis, Italy

INTRODUCTION

Bovine coccidiosis is caused by intracellular protozoan parasites belonging to several species of *Eimeria*. Both clinical and subclinical coccidiosis in young calves results in decreased production from reduced rate of weight gain, efficiency of gain and increased calf morbidity and mortality. The monetary losses due to sub-clinical infection disease may exceed those resulting from clinical coccidiosis (Fitzgerald, 1980) because the former occurs much more frequently and may impair intestinal physiology, feed conversion and growth of animals (Fox, 1985; Matjila and Penzhorn, 2002). In the specific case of the beef cattle production, the disease can be enhanced by any stressful event. *Yucca schidigera* is an herbaceous plant used in traditional medicine by Native Americans to treat a variety of ailments. *Yucca* products are currently used in a number of veterinary applications as feed additives (Cheeke and Otero, 2005) increased growth rate and improved feed conversion efficiency (Mader and Brumm, 1987; Sliwinski *et al.*, 2002), reduction in atmospheric ammonia (Hussain and Cheeke, 1995; McAllister *et al.*, 1998), anti-protozoal activity (McAllister *et al.*, 2001), modification of ruminal microbe

populations (Wallace *et al.*, 1994; Wang *et al.*, 1998, 2000), reductions in stillbirths in swine (Cline *et al.*, 1996), reduction on blood and tissue cholesterol levels in poultry (Oakenfull and Sidhu, 1989) and anti-arthritis activity (Cheeke, 2000). *Yucca* products have GRAS status so are FDA approved for use in humans.

Yucca contains a number of phytochemicals the best known are the steroidal saponins (Oleszek *et al.*, 2001). Saponins have been shown anti-protozoal activity and cause lysis and death by interacting with cholesterol in cell membranes (Wallace *et al.*, 1994; McAllister *et al.*, 2001). This study was conducted to investigate whether extracts of *Y. schidigera* also possess anticoccidial activity when provided as a feed additive and might therefore have potential as an agent for control of oocyst shedding in calves.

MATERIALS AND METHODS

Charolaise/Limousine-cross calves imported from France were stabled in North-Western Italy. Based on common farm procedures at their arrive all the animals underwent to clinical examination to antihelminthic treatment and to vaccination for bovine virus diarrhoea,

bovine respiratory syncytial virus and infectious bovine rhino-tracheitis. At day 0, twenty seven calves (from 7-18 months of age) naturally infected with subclinical coccidiosis, selected for body weight and degree of oocysts shedding were allocated to three groups of 9 animals each. The groups were stabled in similar boxes with the same bedding; no physical contact was possible between calves from different groups. The first group (MON) was fed with a ration containing monensin (Rumensin®100Premix, Elanco Animal Health, Greenfield, IN, USA-140 mg/animal/day); the second group (YS) was fed with a ration containing *Y. schidigera* saponins (MICRO-AID®, DPI, Porterville, 74 CA, USA-15 g/animal/day); the third group (CTRL) was maintained as nonmedicated control.

The three groups were compared during a period of 75 days. Calves were weighted individually at day 0 and at day 75. Cattle were daily monitored for general health status. At day 0, 15, 30, 45 and 75 individual faecal samples were collected from the rectum and the faecal consistency score (1 = liquid, 2 = poltaceous, 3 = normal) was recorded. Calves were shipped for slaughter immediately thereafter (within 7 days). Faecal samples were examined by a modified McMaster technique (Ministry of Agriculture, Fisheries and Food, Great Britain, 1986) and the number of Oocysts Per Gram of faeces (OPG) was recorded. Finally, faecal samples were dried using a forced air oven at 55°C for 48-72, 83 h and the percentage dry matter of faeces was recorded.

The efficacy of the treatments was assessed evaluating the reduction of mean oocyst excretion at each measurement point mentioned above. For each group, the percent oocysts reduction was calculated using the formula; $(100 * (\text{OPG prior treatment} - \text{OPG post-treatment}) / \text{OPG prior treatment})$.

Because of the low number of samples the non-parametric statistic was applied to all data. The Mann-Whitney test was used to assess the differences between groups in the following: OPG values, faecal scores, dry faecal percentages (days 0, 15, 30, 45 and 75); body weight at days 0 and 75.

Means are reported with ± 1 Standard Deviation (SD); statistical significance was established as $p < 0.05$. All statistical analyses were performed with the software R 1.8.0 (Ihaka and Gentleman, 1996).

RESULTS AND DISCUSSION

Both clinical and subclinical coccidiosis in young calves results in decreased production from reduced rate of gain, efficiency of gain and increased calf morbidity and mortality. Severe infections are rare and cause bloody

diarrhea, dehydration, weight loss. In light infections, cattle appear healthy and oocysts are present in normally formed faeces but also in this case feed efficiency is reduced (Waggoner *et al.*, 1994). Assumedly, the monetary losses due to sub-clinical infection disease even exceed those resulting from clinical coccidiosis (Fitzgerald, 1980) because the former occurs much more frequent and may though impair intestinal physiology, feed conversion and growth of animals (Fox, 1985; Matjila and Penzhorn, 2002). In the specific case of the beef cattle production, the disease can be enhanced by stressful event such as the adaptation period also in farms with a good animal management.

As a consequence, routine drug plans are administrated to prevent infections. Compounds recommended for use in controlling or reducing the severity of coccidiosis in calves include quinolones such as decoquinatate and ionophore antibiotics such as lasalocid and monensin (Waggoner *et al.*, 1994). Quinolones present low toxicity in animals but do not allow a total control of the coccidiosis and are often responsible of resistance phenomena (Saitoh *et al.*, 1986). Ionophore antibiotics together with growth-promoting antibiotics used in animal feeds have been moved to prohibit by legislators in Europe (Regulation N. 1831/2003/EC) from the end of 2005 due to the problems concerning the rise of resistance factors that can compromise the potency of therapeutic antibiotics in man.

Furthermore, the increasing consumer demand for organically-produced meat and milk lead to face the problem. Thus, the search continues for a natural alternative for controlling coccidiosis and therefore, to solve problems in animal nutrition and livestock production.

Extracts of *Y. schidigera* have been used *in vitro* (Wang *et al.*, 1998) and *in vivo* (Hristov *et al.*, 1999) saponins have been shown anti-protozoal activity and cause lyses and death by interacting with cholesterol in cell membranes (McAllister *et al.*, 2001; Wallace *et al.*, 1994).

This study was conducted to investigate whether extracts of *Y. schidigera* also possess anti coccidial activity when provided as a feed additive. No abnormal clinical signs were observed in any group throughout the study. No differences in OPG values were observed at day 0 between the three groups. No significant difference was observed in OPG values between MON and YS through the study. On day 15, OPG were significantly lower in YS and MON compared to CTRL (respectively, $W = 11, p = 0.014$ and $W = 14, p = 0.017$). From day 30 to the end of the study, OPG values were similar in all groups

Table 1: Comparison between tested groups

Groups	Weight (kg)			OPG and percent oocysts reduction			
	Day 0	Day 75	Day 0	Day 15	Day 30	Day 45	Day 75
MON	283.6±25.1	413.3±27.6	209.4±79	31.3±5.8 85%	6.3±1.7 97%	6.3±2.1 97%	18.8±4.9 91%
YS	295.9±26	404.6±33	163.1±63	39.9±5.6 75%	26.6±6.7 84%	56.6±10.6 71%	33.3±6.2 80%
CTRL	315.1±23	414.1±29	177.8±62	127.8±37.2 28%	38.9±12 78%	44.4±16.5 75%	22.2± 5.1 88%

(MON = Monensin; YS = *Yucca schidigera*; CTRL = Unmedicated control) on body weight (mean±SD) and OPG values (mean±SD)

and a complete coccidial elimination was not recorded in any group. The highest daily weight gain was recorded for MON (mean daily weight gain = 1.73 kg day⁻¹) with respect to YS (mean daily weight gain = 1.45 kg day⁻¹) and to CTRL (mean daily weight gain = 1.32 kg day⁻¹). The weight recorded at day 0 and at day 75 was significantly different between MON and YS (W = 58, p = 0.04) and between MON and CTRL (W = 57.5, p = 0.04).

Faecal scores and dry-faecal percentages did not differ significantly between groups. Table 1 shows the main results of the trial. The effectiveness of monensin used as a feed additive to protect young calves against *Eimeria* infections has been reported in literature (Fitzgerald and Mansfield, 1973; Goodrich *et al.*, 1984; Bittar *et al.*, 2002). This study suggests that *Y. schidigera* saponins orally administered have an anticoccidial activity similar to monensin one. As reported for *Giardia* (McAllister *et al.*, 2001), anti-coccidial activity of saponins could be due to its ability to destabilize membranes and increase cell permeability by combining with membrane-associated sterols (Price *et al.*, 1987; Gee and Johnson, 1988). Although, *Y. schidigera* saponins as monensin did not completely eliminate coccidia from calves, a reduction in the shedding is important because it may reduce potential environmental contamination.

A greater daily weight gain was observed in animals treated with monensin and with *Y. schidigera* saponins than in control calves. The improved gain by cattle fed diets containing monensin was previously described (Potter *et al.*, 1976; Goodrich *et al.*, 1984) as regard *Yucca saponins* this could be due both to their anti-coccidial activity and selective effects on rumen micro-organisms (Wallace *et al.*, 1994; Wang *et al.*, 1998, 2000) making the best use of nitrogenous resources.

CONCLUSION

The results founded that under conditions of natural exposure to coccidiosis and normal management practices, there was little advantage in gain for medicating calves with *Y. schidigera* saponins compared with nonmedicated control calves.

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