Anticoccidial efficacy and safety of Fytevo®PT alone or in association with Fytevo®AM in naturally infected beef cattle

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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 because the former occurs much more frequently and may impair intestinal physiology, feed conversion and growth of animals. Maslinic acid (2,3-dihydroxyolean-12-en-28-oic acid) is a pentacyclic triterpene found in a variety of natural sources as recently reviewed. Several studies have proved that maslinic acid exerts a wide range of biological activities, i.e. antitumoral, antidiabetic, antioxidant, cardioprotective, neuroprotective, antiparasitic and growth-stimulating. The biological activities of maslinic acid have been assessed in different experimental models, *in vitro* or in animal models, supported by the lack of adverse effects *in vivo* after the oral administration of the triterpene; antiprotozoal activity has been demonstrated *in vitro* against *Toxoplasma gondii* and *Plasmodium falciparum* and *in vivo* in *Gallus domesticus* chicks against *Eimeria tenella* and in mice against *Plasmodium yoelii*. Fytevo®PT is a natural anticoccidial additive that contains steroidal saponins (derived from *Yucca schidigera*, *Taraxacum officinale* and *Panax ginseng*) that has shown anti/protozoal activity. The aim of the present trial was to investigate the effectiveness of metaphylactic treatments with Fytevo®PT alone or in association with maslinic acid (Fytevo®AM) against natural infections by *Eimeria spp.*

MATERIAL AND METHODS
Study site and animals
From May to July 2014 a field study was carried out in an intensive farm in North Western Italy with a history of subclinical and clinical coccidiosis; the farm raises almost 200 Piemontese breed beef calves/year.

SUMMARY
Introduction - Several studies have proved that maslinic acid, a natural pentacyclic triterpene, and Fytevo®PT a natural additive that contains steroidal saponins, and Fytevo®PT a natural additive that contains steroidal saponins, and Fytevo®PT a natural additive that contains steroidal saponins, and Fytevo®AM show anticoccidial activity. Further studies on a larger sample size are needed to confirm the obtained advantage in weight performance of these compounds.

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 because the former occurs much more frequently and may impair intestinal physiology, feed conversion and growth of animals. Maslinic acid (2,3-dihydroxyolean-12-en-28-oic acid) is a pentacyclic triterpene found in a variety of natural sources; as recently reviewed several studies have proved that maslinic acid exerts a wide range of biological activities, *i.e.* antitumoral, antidiabetic, antioxidant, cardioprotective, neuroprotective, antiparasitic and growth-stimulating. The biological activities of maslinic acid have been assessed in different experimental models, *in vitro* or in animal models, supported by the lack of adverse effects *in vivo* after the oral administration of the triterpene; antiprotozoal activity has been demonstrated *in vitro* against *Toxoplasma gondii* and *Plasmodium falciparum* and *in vivo* in *Gallus domesticus* chicks against *Eimeria tenella* and in mice against *Plasmodium yoelii*. Fytevo®PT is a natural anticoccidial additive that contains steroidal saponins (derived from *Yucca schidigera*, *Taraxacum officinale* and *Panax ginseng*) that has shown anti/protozoal activity. Moreover, steroidal saponins enhance rumen fermentations. The aim of the present trial was to investigate the effectiveness of metaphylactic treatments with Fytevo®PT alone or in association with maslinic acid (Fytevo®AM) against natural infections by *Eimeria spp.* in beef calves.
Based on common farm procedures all the animals underwent to anthelmintic treatment and to vaccination for bovine virus diarrhoea, bovine respiratory syncytial virus, Pasteurella and infectious bovine rhino-tracheitis.

**Experimental groups**
At the beginning of the experiment, no animal had received either anticoccidial and/or coccidiostat treatments or had shown any clinical sign of coccidiosis. At study day 0 (SD0), twenty male calves were selected and allocated to five balanced groups of four animals each, according to their age (10.9±0.66 months) and live weight (453.7±28.38 kg). The groups were stabilized in similar pens (3 x 6 m) with the same bedding and diet; no physical contact was possible among calves from different groups.

Animals were fed ad libitum. The ration of four groups were daily supplemented with Fytevo®PT 500 ppm (Group A), Fytevo®PT 1000 ppm (Group B), Fytevo®AM 125 ppm (Group C), Fytevo®PT 1000 ppm + Fytevo®AM 250 ppm (Group D), respectively; the last group (Group CTRL) remained untreated control.

**Parameters**
The five groups were compared during a 80-day experimental period.
Calves were daily monitored for general health status; individual body weight was quantified at SD0 and at SD80 by a weighing scale, calibrated at each use. Separate working clothes, footwear and equipment were used for each pen throughout the experimental period in order to avoid cross-infection among groups.

At SD0 and weekly for 8 follow-ups (until SD67) individual faecal samples were collected weekly from the rectum and examined for i) faecal consistency score (1 = liquid/haemorrhagic faeces, 2 = poltaceous or soft faeces, 3 = normal consistency), ii) pH of faeces, iii) number of oocysts per gram of faeces (OPG) by modified McMaster technique. Finally, faecal samples were dried using a forced air oven at 55°C for 24 h and dry matter (%) of faeces was recorded.

At the end of the trial (SD80), pool faecal sample for each experimental group were collected and analysed as above; moreover the gross chemical composition of each pool was determined.

**Data analysis**
The statistical analysis of the data was performed using the SAS 9.1.3 software. The efficacy of the treatments was assessed by calculating the percentages of Faecal Oocyst Count Reduction (FOCR) at each measurement point mentioned above, according to the following formula: \[ \text{FOCR} = \left( \frac{\text{OPG prior treatment} - \text{OPG post treatment}}{\text{OPG prior treatment}} \right) \times 100\% \].

The number of oocysts per gram of faeces (OPG) were log-rhythmically transformed and added by 1 \[ \log(\text{OPG} + 1) \] in order to obtain normal distributions according to Kolmogorov-Smirnov’s normality test. Gross chemical composition of faeces, body weight, and average daily gain data were subjected to one-way analysis of variance (ANOVA). Related post-hoc comparisons were performed using Tukey or Mann-Whitney U tests.

**Ethical standards**
All procedures were carried out in compliance with current Italian laws and European guidelines.

**RESULTS**

**Number of oocysts per gram of faeces (OPG)**
At the beginning of the study, no statistically significant differences were observed among groups with regards to age, oocysts excretion, body weight or faecal parameters.

Oocysts were identified as *E. bovis*, *E. auburnensis* and *E. ellipsoidalis* based on published oocyst descriptions; at SD0 OPG values were 1850±75 in Group A, 2075±88 in Group B, 1800±40 in Group C, 1860±65 in Group D and 1750±60 in Group E.

Figure 1 shows means (±SD) logarithmically transformed *Eimeria* spp. oocysts excretion at each experimental time.
point in the five experimental groups. Starting from SD7 the percentage oocysts reduction of Group C (16.67%) and Group D (10.00%) were significantly lower compared to other groups (Group CTRL: 1.35%; Group A: 4.82%; Group B: 3.39%); from SD14 to the end of the trial the four treated groups showed no statistical differences, ranging from 64.30%–74.40% (SD14) to 95.40%–97.00% (SD80). The highest percentage oocyst reduction of Group CTRL was recorded at SD80 (35.14%).

Body weight and average daily gain
Animals in Group C showed a higher average daily weight gain of 1,610±330 g compared to Group A (1,470±410 g), Group B (1,430±120 g), Group D (1,380±340 g) and CTRL (1,230±150 g), respectively. However, the statistical analysis showed no differences among groups.

Faecal dry matter
The average percentage of faecal dry matter did not show significant variations among the five groups until SD29. From SD37 to the end of experimental trial the dry matter content in the faecal samples of Group D was significantly lower compared to the other groups (SD37: 15.25±2.50; P<0.05; SD45: 13.50±2.38; P<0.001; SD59: 14.75±3.10; P<0.01; SD67: 12.25±2.35; P<0.001).

Faecal pH
Up to SD21, faecal pH values did not significantly differ among the five experimental groups, ranging from 6.64 to 7.26; starting from SD29 to the end of the trial Group D showed pH values significantly lower (always for P<0.01) compared to other groups (SD29: 6.37±1.19; SD37: 6.21±0.103; SD45: 6.06±0.124; SD59: 6.48±0.192; SD67: 6.22±0.171; SD80: 6.24–pool value). pH mean values at each time point in the five experimental groups are reported in Table 1.

Faecal score
A similar trend observed for faecal pH was observed also for faecal scores. From SD29 to the end of the trial Group D showed values significantly (P<0.05) lower compared to the other groups, always showing average values greater than 2.75. Furthermore, from SD45 two animals of Group D showed subclinical signs of acidosis and the whole Group showed a strong modification of the faecal aspect, with presence of undigested feed in stool samples (Figure 2).

<table>
<thead>
<tr>
<th>SD</th>
<th>CTRL</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>SEM</th>
<th>P</th>
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<tr>
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<td>7.01*</td>
<td>7.02*</td>
<td>6.78*</td>
<td>6.21*</td>
<td>0.103</td>
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<tr>
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<td>6.95*</td>
<td>6.89*</td>
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<td>6.48*</td>
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<tr>
<td>67</td>
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<td>6.90*</td>
<td>7.11*</td>
<td>6.22*</td>
<td>0.171</td>
<td>**</td>
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</table>

CTRL: untreated group; Group A: Fytevo®PT 500 ppm; Group B: Fytevo®PT 1000 ppm; Group C: Fytevo®PT 500 ppm + Fytevo®AM 125 ppm; Group D: Fytevo®PT 1000 ppm + Fytevo®AM 250 ppm. (*) P< 0.05 and (**) P< 0.01 represent significant differences among groups; ns: not significant. **Means within a row with different superscripts differ significantly.

Figure 2 - Undigested feed in stool samples. Comparison at SD45 between A (CTRL Group, average faecal score: 3±0) and B (Group D, (Fytevo®PT 1000 ppm + Fytevo®AM 250 ppm, average faecal score 1.50±0.41).
Table 2 - Gross chemical composition of faeces in the five experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>CTRL</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<td>DM (%)</td>
<td>20.51</td>
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<td>1.84</td>
<td>2.54</td>
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<td>EE (%DM)</td>
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<td>1.40</td>
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<tr>
<td>NDF (%DM)</td>
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<td>40.98</td>
<td>56.14</td>
<td>57.29</td>
<td>80.28</td>
<td>5.822</td>
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</table>

CTRL: uninfected group; Group A: Fytevo®PT 500 ppm; Group B: Fytevo®PT 1000 ppm; Group C: Fytevo®PT 500 ppm + Fytevo®AM 125 ppm; Group D: Fytevo®PT 1000 ppm + Fytevo®AM 250 ppm. DM, dry matter; EE, ether extract; NDF, neutral detergent fiber. (*) P<0.05 represents significant differences between groups. ** Means within a row with different superscripts differ significantly.

Gross chemical composition of faeces

Values of gross chemical composition of faeces sampled at SD80 are presented in Table 2. These values confirmed what was previously observed in the Group D, showing a significant (P<0.001) lower dry matter content compared to the other groups. Furthermore, the other parameters showed statistically differences among the experimental groups, showing higher amounts of undigested components, particularly in the Group D.

Mean gross chemical composition of faeces at each time point in the five experimental groups are reported in Table 2.

DISCUSSION

Bovine coccidiosis is caused by more than 20 Eimeria spp., most of which are considered harmless. In particular two species, E. bovis and E. zuernii, are pathogenic to calves, while other species such as E. auburnensis and E. ellipsoïtdalis have also been occasionally observed to cause diarrhoea. Subclinical coccidiosis occurs much more frequent than clinical coccidiosis, resulting in decreased production from reduced feed conversion and growth of animals; furthermore, the disease can be enhanced by stressful event, such as the adaptation period also in farms with a good management.

As a consequence, to prevent losses due to coccidiosis, exposed calves are treated prophylactically rather than therapeutically. A series of effective drugs is available, classified according to their apparent mechanisms of action. The advantage of drug medication is its wide action spectrum, i.e., drugs are widely applicable against many different Eimeria species; a major disadvantage, however, is drug resistance as an ever existing and widely occurring problem. Moreover, there are increasing concerns about the impact of widely used drugs on the food chain and environment, all the more as accessible information hereof is scarce. Thus, there is an urgent need to search for novel active agents characterized by lower risks for consumer health and by more environmental compatibility and sustainability. This study was conducted to investigate the effectiveness of metapyllactic treatments with Fytevo®PT alone or in association with maslinic acid (Fytevo®AM) against natural infections by Eimeria spp. in beef calves. As previously reported in chickens and calves, the anticoccidial efficacy of Fytevo®AM and Fytevo®PT was confirmed in the present study by the reduced number of oocysts recovered from calves’ faeces.

Due to their well-known mechanisms on protein-turnover rates (maslinic acid) and rumen fermentations (steroidal saponins), the two natural compounds have been suggested as potential factors to ameliorate animal performance. However, effects on animal performance of maslinic acid were reported in fishes only. Concerning steroidal saponins, increased performance were obtained in broiler chickens and preliminary results were observed in calves. To the best of our knowledge, no previous studies investigated the combined effect of maslinic acid and steroidal saponins on anticoccidial efficacy and growth performance.

Reduction of consistency score, pH, and dry matter content in faecal samples of group D could suggest modifications of rumen activities. Yucca extracts are known to enhance starch-digesting bacteria while inhibiting the fibrolytic bacteria in the rumen, thus favouring the growth of lactic bacteria and an acidification of rumen pH. The higher contents of undigested feed components in the faecal samples collected at the end of the experimental trial confirm a probably compromised rumen activity in the Group D. However, possible synergic effects in the rumen of steroidal saponins and maslinic acid when supplemented over a threshold level need further investigations.

In conclusion, the study suggests that Fytevo®PT, alone or in association with Fytevo®AM, shows anticoccidial activity; further studies on a larger sample size are needed to confirm the obtained advantage in weight performance.

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