



## Supplementation of moist and dehydrated citrus pulp in the diets of sheep artificially and naturally infected with gastrointestinal nematodes on the parasitological parameters and performance<sup>☆</sup>



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### ABSTRACT

The inclusion of industrial byproducts such as citrus pulp in the composition of animal diets has been widely recommended due to sustainability aspects and their high level of carbohydrates. Limonene is found in citrus pulp and has been described elsewhere as a major compound of citrus essential oils with excellent anthelmintic activity. The objective of this study was to evaluate the parasitological parameters of lambs artificially infected (Experiment 1) with *Haemonchus contortus* and naturally infected (Experiment 2) by gastrointestinal nematodes, fed diets with dehydrated citrus pulp or silage of moist orange pulp. Both experiments had three treatments (C: control, DP: diet+dehydrated citrus pulp, and MP: diet+silage of moist orange pulp). The diets were isoproteic (11% crude protein) and the concentrate was corrected every 14 days according to animal weight. Parasitological parameters were evaluated for both experiments each 14 days (body weight, body condition; fecal egg counts-FEC, egg hatch assay-EHA, coproculture, and packed cell volume-PCV). Analysis of variance (GLM of the SAS software) was performed with repeated measures in time, and the means were compared by the Tukey test. Gas chromatography with mass spectrometry was used to detect constituents of dry or moist citrus pulp. Dehydrated citrus pulp had 0.02% essential oil (major compounds were 85.9% limonene and 7.6% valencene). Moist orange pulp contained 1.5% essential oil (major compounds were 65.5% limonene and 31.2% alpha- and gamma-terpineol). In both experiments, the weight gain among the treatments was similar ( $p > 0.05$ ) demonstrating that both moist and dehydrated orange pulp can be used to replace corn kernels to feed infected lambs. The supplementation with orange pulp did not decrease natural or artificial infections of gastrointestinal nematodes according to the FEC results ( $p > 0.05$ ). However, PCV increased from animals fed dehydrated and moist pulp in natural infection (Experiment 2,  $p < 0.05$ ) in comparison

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with the control group. In addition, the consumption of the dehydrated citrus pulp from animals infected with *H. contortus* (Experiment 1) caused lower hatching rates after 42 days of consumption ( $p < 0.05$ ), suggesting a tendency to shed fewer eggs to the environment.

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## 1. Introduction

The commercial anthelmintics used to treat gastrointestinal nematode infections in ruminants are largely limited to three classes of drugs: benzimidazoles, imidazothiazoles/hydropyrimidines and macrocyclic lactones, for which parasitic resistance is already widespread. Three highly effective drugs have recently been launched, emodepside, monepantel and derquantel, but they are fairly expensive. Thus, the development of resistance seems to outpace the introduction of new anthelmintic drugs (El-Abdellati et al., 2010; Buttle et al., 2011). The detection of active substances in plant extracts can help control parasites, reduce veterinary drug residues in animal products and prolong the useful lifetime of the commercial products (Chagas, 2008). The moist and dehydrated pulps of citrus fruits can act as an eventual anthelmintic since they contain essential oils with large quantities of terpenes (Klavons et al., 1994), which are secondary metabolites that interfere in the biochemical and physiological functions of parasites (Rice and Coats, 1994).

Besides these aspects, the use of byproducts or residues from food processing in animal feed can be beneficial because as well as diminishing production costs, this practice can reduce environmental waste (Dutra et al., 1997). Brazil is responsible for nearly 90% of the oranges grown in South America and 34% of global output, with yearly national production of around 19.8 t (IBGE, 2011). After extraction of the juice, the moist pulp, composed mainly of peel fragments, membrane pieces and seeds, accounts for 44 to 50% of the original fruit weight (Widmer et al., 2010). This pulp, containing approximately 82% water, needs to be disposed of adequately, because industrial wastes cannot be accumulated indefinitely at the place of production (Pelizer, 2007).

Various experiments have demonstrated the nutritional and pharmacological (or nutraceutical) benefits of secondary metabolites from plants, such as improved performance of livestock. An experiment demonstrated that the inclusion of fresh orange pulp in sheep diets resulted in greater dry matter and nutrient intake and promoted better nitrogen use and greater apparent digestibility compared to diets with sorghum silage (Macedo et al., 2007). According to Callaway et al. (2011), the pulp and peel of oranges and other citrus fruits contain essential oils (e.g., limonene) that are toxic to bacteria and exhibit antioxidant effects in host animals. They further reported that feed composed of 10% orange peel reduced *E. coli* O157:H7 and *Salmonella* throughout the gastrointestinal tract of sheep used as model ruminants. Squires et al. (2010) reported a decline of 97.4% when limonene was administered at a dose of 600 mg/kg LW. Since in this study the essential oil was consumed over a longer period, we expected to obtain significant results regarding the metabolic pathways,

survival or hatching rate of the nematodes. Therefore, the aim of this study was to evaluate the effect of supplementation with dehydrated orange pulp and silage of moist orange pulp on the parasitological parameters of lambs infected naturally and artificially by gastrointestinal nematodes.

## 2. Material and methods

This study was approved by the institute's ethics committee (finding no.142/2011).

### 2.1. Locale

The experiments were conducted at Instituto de Zootecnia/APTA/SAA, located in São Paulo state, southeastern Brazil (22°42'S and 47°18' W). The area's climate is Cwa (Köppen), characterized by rainy summers and dry and mild winters. The two experiments were simultaneously conducted between June and August 2012, during the dry season with mean temperature of 18.4 °C.

### 2.2. Animals

Sixty-nine Santa Ines ewe lambs were used: 24 lambs in Experiment 1 (artificial infection); and 45 lambs in Experiment 2 (natural infection), with ages of 3 to 4 months.

### 2.3. Experiment 1—Animals confined and artificially infected with *H. contortus*

Twenty-four animals with LW of 20 kg ± 1.4 were administered trichlorfon (Neguvon®, Bayer, 1 mL/kg of a 10% solution). After 28 days and measurement of FEC through three consecutive counts of zero, each animal was inoculated with 3500 L<sub>3</sub> of *H. contortus* isolate Embrapa 2010, provided by Embrapa Pecuária Sudeste (Southeast Livestock Research Unit of the Brazilian Agricultural Research Corporation), characterized as resistant to benzimidazoles, macrocyclic lactones and imidazothiazoles (Chagas et al., 2013). After 28 days, the animals were weighed and FEC was performed to confirm the infection, after which the animals were divided into three treatment groups, blocked according to the mean FEC and assigned to individual pens (8 pens per group). The treatments were:

**CONTROL (C)**, corn silage *ad libitum* and concentrate composed of ground corn kernels, soybean meal and mineral mix (1% of the animals' LW);

**DEHYDRATED CITRUS PULP (DP)**, corn silage *ad libitum* and concentrate composed of dehydrated citrus pulp, soybean meal and mineral mixture (1% of LW);

**MOIST ORANGE PULP (MP)**, corn silage *ad libitum* and concentrate composed of soybean meal and mineral

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