Molasses and maize against GI nematodes

*Evaluating the effect of molasses and maize
on the resilience and resistance
against GI nematodes in browsing Criollo kids*

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ABSTRACT

Gastrointestinal parasites are a major problem for the holders of small ruminants in the tropics and sub-tropics. Anthelmintics have been used and abused to such an extent that a high degree of resistance has developed in many countries. There is urgent need for alternative control methods, and studies take place all over the developing world to find such methods.

The Autonomous University of Yucatán, in Mérida, Mexico is a place where these alternative methods of control are under study. The objective for my visit there was to be involved in an investigation concerning one of the promising control methods, namely supplementary feeding of goats browsing native vegetation.

My participation in this project was made possible by the Minor Field Study (MFS) program in cooperation with the Swedish University of Agricultural Sciences (SLU). The report was originally written as an MFS.

Picture 1. La Sucia, one of the investigation goats.

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Abbreviations

AH	Anthelmintics
COWP	Copper Oxide Wire Particles
EPG	Eggs Per Gram Faeces
FEC	Faecal Egg Count
FMVZ-UADY	Faculty of Veterinary Medicine and Animal Science of the Autonomous University of Yucatán
GIN	Gastrointestinal nematodes
ME	Metabolisable Energy
PC	Crude Protein
PCV	Packed Cell Volume
S	Sampling event
SVA	The National Veterinary Institute, Sweden

PREFACE

Anthelmintic resistance amongst nematode parasites of livestock is a big problem for holders of small ruminants in many parts of the world. Feed supplementation has proven to be a good method to limit the adverse effects of parasites in animals. Earlier studies with sheep and goats have shown that sorghum and soybean meal are effective supplementations, and maize has proven to be almost as effective. Because maize is useful in human nutrition, and expensive, it cannot be considered a good choice in the long term as an animal feed. In this investigation, molasses was tested to determine whether it could replace maize as a feed supplement for goats.

This report is a result of a Minor Field Study, carried out in Mexico during September through December 2005. The investigation started in August and ended in December, shortly after I had left Mexico. My local supervisor was Felipe Torres-Acosta, FMVZ-UADY, Mexico and my Swedish supervisors were Johan Höglund, and Peter Waller at SLU/SVA, Sweden.

BACKGROUND

1. Small ruminant production

The goat has been called the poor man's cow for its ability to provide sufficient meat, milk and fibre for a farmer's own use, with perhaps a little left to sell (Greve-Isdahl, 2003). Small ruminants (sheep and goats) are very important livestock to smallholder farmers in the developing countries of the world. However, because most of the farmers that keep goats are resource-poor, goat production has been a neglected issue(Steele-96).

In Mexico the population of small ruminants is approximately 16 million of which approximately 6,5 million are sheep and 9,5 million are goats (SAGARPA, 2001). The market for the holders of small ruminants in Mexico is difficult because of competition from, for example, Australia, New Zealand and USA.

On the Yucatán peninsula in eastern Mexico, farmers keep their small ruminants in semi-intensive systems where the animals are confined at night but are allowed to browse daily. Overall 73 % of the animals receive supplementation, mainly in the form of maize, soybean meal and local vegetation. Complete stabling and intensive systems are rare. Approximately 60 % of the farmers admit to having problems with diarrhea among their animals, and 75 % claim that gastrointestinal (GI) parasites cause them trouble by making the animals thin and weak, and sometimes killing them (Torres-Acosta, 1999).

2. The parasite problem

2.1 Gastrointestinal parasites

Nematodes and in particular the abomasal worm *Haemonchus contortus*, are the biggest threat to small ruminant stockholders in the tropics and subtropics. They cause great losses in production because of poor weight gain, decrease in milk production and sometimes even death of the animals. Other common GIN in goats on the Yucatán peninsula, are ; *Trichostrongylus colubriformis* (small intestine), , *Oesophagostomum columbianum* and *Trichuris spp* (large intestine) (Basulto 2000, Uitz 2001). *Strongyloides papillosus* is also present in the small intestine..

2.2 The life-cycle of the GIN

The parasite eggs are passed out into the environment within the faeces of the host. The L1 (first larval stage) develops within the egg and under ideal conditions hatches within 24 hours. The L1 actively feeds on bacteria within the faecal pat, undergoes exsheathment to become the second stage larvae (L2). After about 1 week it develops into the third stage larvae (L3), retaining the second stage cuticle. The L3 stage cannot feed and is the infective stage. These larvae are ingested by the browsing animal and then mature into adult parasites after about 3 weeks in the gastrointestinal tract. If the environment does not suit the parasite's development it is possible for it to enter a hypobiotic phase in the tissues until more suitable conditions are encountered (Urquhart, 1996).

2.3 Factors that influence the level of GIN in an animal

2.3.1 Internal factors

- Goats are more susceptible than sheep to GIN (Soulsby, 1987).
- Crosses between goat breeds tend to have stronger resistance against parasites than pure breeds.
- Native animals survive better than imported breeds because of their environmental adaptation.

All animals need to be in a good nutritional state to be able to provide enough protein to repair tissues, produce anti-bodies and induce an immune response to infection.

2.3.2 External factors

The optimal temperatures for the development and survival of the free-living stages on pasture are between 22-26 degrees Celsius (Hansen and Perry, 1994) and a humidity of 100 % (Urquhart, 1996). Therefore the amount of larvae on the pastures increases during prolonged rain periods. Many larvae then resume in development from the hypobiotic state they were in during the dry period (Hansen and Perry, 1994). Adult parasites can also sometimes survive from one wet season into another. Some infective larvae can survive inside the faecal pellets out on the pastures (Torres-Acosta, personal communication, 2005).

2.4 Pathologic effects of the GIN

The most pathogenic types of GIN of goats on the Yucatán peninsula are those listed below.

2.4.1 Haemonchus contortus

Adult worms causes anemia when, in the case of a bad nutritional state or during heavy infections with this parasite, its ingestion of blood in the abomasum exceeds the hematopoesis (Urquhart 1996).

2.4.2 Trichostrongylus colubriformis

Causes loss of blood plasma because it destroys the crypts of Lieberkühn in the small intestine and thereby causes increased vascular permeability and loss of epithelia (Sykes 1979, Steel et al., 1982, Hosté 2001).

2.4.3 Oesophagostomum columbianum

The migrating larvae cause hyperplasia of the mucosa and therefore easily cause diarrhea and sometimes colitis (Blood and Radostitis, 1992). The nodules induced by larvae penetrating the gut mucosa may rupture, causing adhesions or obstructions. The parasite may also cause hypoproteinemia, edema, and thickening of the wall of the large intestine and the caecum.

2.5 Clinical findings in cases of GIN infection (nematodosis)

The most important clinical signs are loss of appetite, diarrhea, sometimes subcutaneous edema and a bad general state of health (Eysker and Ploeger 2001). As indicated above *H. contortus* causes anemia and therefore pale mucous membranes. The damage done by the parasite causes weight losses, and hence economical losses to the farmer.

Subclinical nematodosis is the most common form, the acute form is mostly found in young animals. The acute signs are those of anemia through pale mucous membranes and low hematocrit levels (less than 12%). The animals also often have sub-mandibular and/or ventral edema. Diarrhea is often only seen when the infection is combined with *T. colubriformis* infection. If *H. contortus* is the only parasite species present, this normally results in dry faeces. If the *H. contortus* burden is very heavy, animals can be found dead without prior warning (Abbot et al, 1985).

2.6 Diagnostic techniques

- *Coprologically*: McMaster and flotation techniques. The flotation only indicates the presence of parasites (qualitative) while the McMaster technique estimates the level of infection (quantitative). Coprocultures are used to determine the genus of nematode present in the faeces of infected animals.
- *Postmortem:* Necropsy can be used to prove the existence of adult worms, the size of the infection and the species involved in such infection.
- *Miscellaneous:* Recovering infective larvae from pasture by various pasture sampling techniques, or using the tracer kids/lamb technique to determine the infection status of the pasture for a given period of time (generally between 2 and 4 weeks). Other techniques include measuring the amount of pepsinogen in the blood and specialized serology (Torres-Acosta, 1999)

Picture 2. The McMaster technique.

3. Control methods

3.1 Methods of controlling the GIN

- *Interfering* with the lifecycle of the parasite by means of conventional anthelmintics, using rotational grazing or stabling, biological control with nematophagous fungi, non conventional anthelmintics such as COWP boluses and tanniniferous plants. (Torres-Acosta, 1999).
- *Strengthening* the animal through vaccination, choosing genetically resistant animals and/or by giving supplementary feeding.

3.2 Conventional methods

3.2.1 Anthelmintics: Advantages and disadvantages

The advantage of using anthelmintics (AH) is that the animal responds quickly and the symptoms generally vanish rapidly. One of the many disadvantages is that AH are expensive (Mendoza, 2000). Irrational use of wide spectrum AH has lead to severe resistance against AH in the worm populations, and once the resistance problem is present there is little chance of it being successfully reversed (Waller, 1997).

3.2.2 Anthelmintic resistance

The resistance is a reduction in the effectiveness of anthelmintics and is genetically inherited in a population of parasites. Resistance develops when AH are used too often, at the wrong dose or when the wrong type is used. In many parts of the world parasite control has largely been based on AH for decades and this has lead to widespread development of anthelmintic resistance (Waller, 1987). A worrying example is that in Yucatán, Mexico, the prevalence of goat herds with benzimadazole resistant nematodes is 57% (Torres-Acosta et al, 2003).

3.3 Alternative control measures

Alternative methods can be used, such as a prophylactic worm control, but does not substitute the use of AH, they just reduce the need for treatment. This is not to say that the alternative methods are useless, but they should be combined with a timely and effective use of AH (Waller, 2003).

3.3.1 Stabling and rotational grazing

Stabling the animals is not a possible solution for smallholder farmers in Yucatán, Mexico. The work and money invested in feeding stabled animals would not be affordable in the long run.

Rotational grazing is a very efficient way of keeping the parasite burden low and reduces the need for AH. This way of helping the animals against the parasites should be given more attention among the farmers, even though it takes time and effort to put into practice. There is a need to determine if such methods of control are viable under the tropical conditions of Yucatán as this area is not as humid as the places where it has been proved efficient (Fiji: Barger et al., 1994; Malaysia: Chandra watani et al., 2004).

3.3.2 Nematode trapping fungi

Studies done with nematode trapping fungi, such as *Duddingtonia flagrans*, has shown that this could be a reasonable alternative. When given in high doses it is efficient in trapping nematode larvae in faeces. *D. flagrans* survives the gut passage without loosing viability and can therefore easily be added to processed food given to the animals. The spores can also be given as intraruminal controlled release device tablets, or as feed blocks (Chandrawathani, P. 2004).

3.3.3 Tanniferous plants

Positive results have also been achieved in studies dealing with tanniferous plants and tannins given to sheep and goats as anthelmintics, but these indications are yet to be confirmed in the field (Hosté, 2000).

3.3.4 Copper Oxide Wire Particle (COWP) Boluses

Copper is an alternative tool that can be given in the form of copper oxide wire particles (COWP) enclosed in a capsule. The capsule is given orally and releases the copper wire particles in the rumen which then lodge in the mucosal folds of the abomasum, where they slowly dissolve (Bang et al., 1990). Several investigations show that the COWP reduces the EPG-excretion of *H. contortus*, but not when copper is given alone (Vargas-Magaña et al, 2003).

An investigation carried out by Vargas-Magaña et al (2003) showed that COWP, combined with supplementation, improved resilience to parasitism in goat kids by increasing the weight gaining, the Hb and the packed cell volume (PCV), and to a certain degree the resistance (measured by EPG and peripheral eosinophil counts).

Recent work in goats and hair sheep in Yucatán has demonstrated a sharp reduction (60 to 90%) in the *H. contortus* infection during a period of 3 to 4 weeks. However, such reductions are seldom accompanied by an improvement in resilience, as the other worm populations (*T. colubriformis* and *O. columbianum*), are equally capable of reducing productivity in naturally infected animals. Investigations show that goats are less susceptible to copper intoxication than sheep and are therefore better suited to use COWP. More investigations focusing on the usage of COWP on goats should be undertaken before ascertaining COWP as a reasonable alternative measure. (Canto-Dorantes et al., 2005; Perez-Garcia et al., 2004; Zaragoza-Vera, 2005).

3.3.5 Vaccines

There are not yet any commercially available vaccines for the control of helminth infections in ruminants, with exception of Dictol®, a vaccine against the bovine lungworm, *Dictyocaulus viviparus*.

Researchers have used proteins extracted from the surface of the intestinal cells of *H. contortus* and used to immunise sheep. The results are promising but a big problem is that round worms such as *H. contortus* cannot be cultured, it can only be obtained from infected animals. This makes the development of the vaccine very expensive and not cost effective enough. (Smith, 2007)

3.3.6 Supplementary feeding

Supplementation is needed to secure sufficient nutrients to repair tissues in case of normal or pathological deterioration. It is also beneficial for securing a normal blood production and a sufficient immune defense. The supplementation counter-acts the maturing and survival of parasites, as well as the fecundity of the parasite females, but it does not affect the first phases of parasite infection (Coop and Kyriazakis, 1999).

The most investigated form of supplementary feeding is in the form of protein supplementation given daily. This method is based on evidence indicating that such supplementation helps the animals to withstand the pathogenic effects of the parasites. (Van-Houtert and Sykes, 1996). It also assists animals in maintaining their level of growth and production even when burdened with parasites (i. e. *resilience*). It also permits a sufficient immunological response to regulate the parasite population (i.e. *resistance*) (Torres-Acosta, 1999).

The supplementation with rumen fermentable energy improves the utilization of the dietary protein, improving both resistance and resilience in the animal. The energy can be used by the ruminal microbes that produce protein (Hosté et al., 2005). A recent study in Mexico showed that a daily supplementation of soybean meal (26 %) and sorghum (74 %) significantly increased the resilience against GIN in Creole goats, but it did not affect their resistance (Torres-Acosta et al., 2004).

Gutiérrez et al., (2002) demonstrated that the use of 108 g of milled corn enhances the utilization of dietary protein from normal grazing of local vegetation to even fulfill the growth requirements of animals. For nitrogen to be sufficiently used in the rumen it is necessary to have a source of dietary energy, e.g. from corn. The disadvantage of using this precise ingredient is that it is not economical and that it is a very important source of energy for the Mexican people. It is therefore necessary to look for alternative sources of rumen fermentable energy. Molasses is a good example because of its low cost and because it is fermentable in the rumen. It can offer benefits similar to those of corn in animals browsing local vegetation.

THE INVESTIGATION

4. Components of the investigation

4.1 Objective

To investigate if molasses is an acceptable alternative to maize as a supplement for Criollo kids during the wet season. Parameters for resilience and resistance against GI nematodes were taken into account.

4.2 Location and timing

The investigation was carried out on the production area of the FMVZ-UADY research facility at Xmatkuil, in the community of Mérida, Yucatán. The study took place during August through December 2004, during the wet season.

4.3 The Yucatán climate

The climate is sub-tropical, sub-humid with a dry period extending through December to May and a rain period between June and November. The annual rainfall is 940-1100 mm with 70 % of the rain falling during the rain period, and the annual average temperature is 26 °C (Garcia, 1973).

Picture 3. Two goats in individual pens being fed maize.

4.4 Animals in the investigation

A group of 42 Criollo kids with an average weight of 17.0 kg, with an average age of 8 months were selected. The kids were brought up free from GIN using the methodology described by Torres-Acosta (1999).

Table 1. Animals in the investigation

Group	Animals	Treatments
T-Mo	8	Treated with moxidectin (AH) and supplemented with molasses.
T-Co	8	Treated with moxidectin (AH) and supplemented with corn.
NT-Mo	13	Not treated, supplemented with molasses.
NT-Co	13	Not treated, supplemented with corn
Total	42	

4.5 Supplementation

The kids in the T-Mo and NT-Mo groups were supplemented individually with 150 g of molasses daily when returning from grazing during the afternoon. The kids forming the T-Co and NT-Co groups were equally supplemented with 108 g of chopped corn.

Table 2. Nutritional facts about the supplementation

Ingredients	Metabolisable energy (ME) / kg	Crude protein (CP) / kg	Amount of food	ME per portion	CP per portion
Molasses	12 MJ	30 g	150 g	1.69 MJ	4.23 g
Maize	14.2 MJ	80 g	108 g	1.53 MJ	8.64 g

4.6 AH treatment

The kids in the T-Mo and T-Co groups were treated day 0, and thereafter every 28 days, with injectable moxidectin (Cydectin, Ford Dodge). It was sub-cutaneously applied using a dose of 0.2 mg/kg live weight.

4.7 Pasturage and feeding

The animals were held under semi-extensive conditions, allowed to browse the native vegetation six hours per day. Shepherds looked after the animals during the browsing period, which was between approximately 7:30 am and 13:30 pm each day. The exact time of browsing was registered each day to make sure that the experimental conditions were adhered to. The animals received their supplementation when returning from the daily browsing. During the night the goats were kept in a pen that held 42 individual cages.

4.8 Sample taking

Day 0 and every 14 days the goats were weighed and samples of blood and faeces were taken from every animal. This took place at 7:00 am and the weighing therefore took place 18 hours after the last feeding.
a) **Blood** 3 ml of blood was taken from each goat through the jugular vein, using individually marked test tubes with EDTA.

b) **Faeces** Approximately 5 grams of faeces was collected directly from the rectum of each animal, using polyethylene bags marked with the number of each animal.

4.9 Short about methods

The following factors were tested to determinate the resistance and resilience of the goats against GIN. The techniques are more thoroughly described under Methodology.

4.9.1 Resistance

- Parasite burden, i.e. FEC or EPG, was measured by using the McMaster method.

4.9.2 Resilience

- Changes in live weight were measured by using a normal pair of scales.
- The hematocrit (Ht) was measured by using the capillary microhematocrit technique (Benjamin, 1991).

4.9.3 Statistical analysis:

The growth rate of the different groups, the Ht value and the FEC (i.e. EPG) were compared by means of repeated ANOVA measures using general lineal models with the SPSS program. FEC was analysed with the same strategy but the FEC was transformed to logarithms before analysis (log 10 (FEC+1)).

4.10 Problems during the experiment

An outbreak of contagious ecthyma (CE, also called orf) was discovered on the 22nd of September. The disease spread to the whole group, affecting all the animals to different degrees. Blisters in the mouth area made it painful for the animals to eat. The goats being fed molasses were less affected than the ones being fed corn because the molasses could be licked when eaten - the corn had to be chewed. This clearly affected their weight gains. The goats recovered from the orf about three weeks before the investigation ended.

Some of the goats failed to consume their entire supplementary ration. These animals were only in the group fed corn. Residues were weighed and registered, however none of the goats were eliminated from the investigation because of their habit of leaving food.

5. Results and Discussion

5.1 Weight gain

There was no significant difference (P<0,05) in weight-gain between the T-Mo and the T-Co groups, thus, maize and molasses showed to be equally efficient when the animals were treated with AH. Similarly, NT-Co and NT-Mo groups gained weight similarly (P<0.05). However, both the non-treated groups had slower growth rates compared to treated groups. This was the direct reflection of the natural infection in the non-treated groups. According to these results, infected animals eating molasses or maize equally improved their resilience against GIN.

However, this trial shows again that the sole use of supplementary feeding cannot overcome completely the negative effects of GIN infection. Thus, there was a reduction in growth rate due to such infection as has been reported in earlier trials (Torres-Acosta et al., 2004). Such effect may suggest that the level of supplementation should be increased in order to increase the nutrients available for the animals in the case of infection.

It is possible that the NT-Mo group recovered faster than NT-Co group after the orf outbreak because of the supplement was much less painful to consume. Orf affected all the kids between sampling times 6 or 7 (S6-S7). Although the animals were infected at almost the same time, their rate of recovery differed. The immune status of each animal also decided how quickly they recovered and when they could start eating normally again.

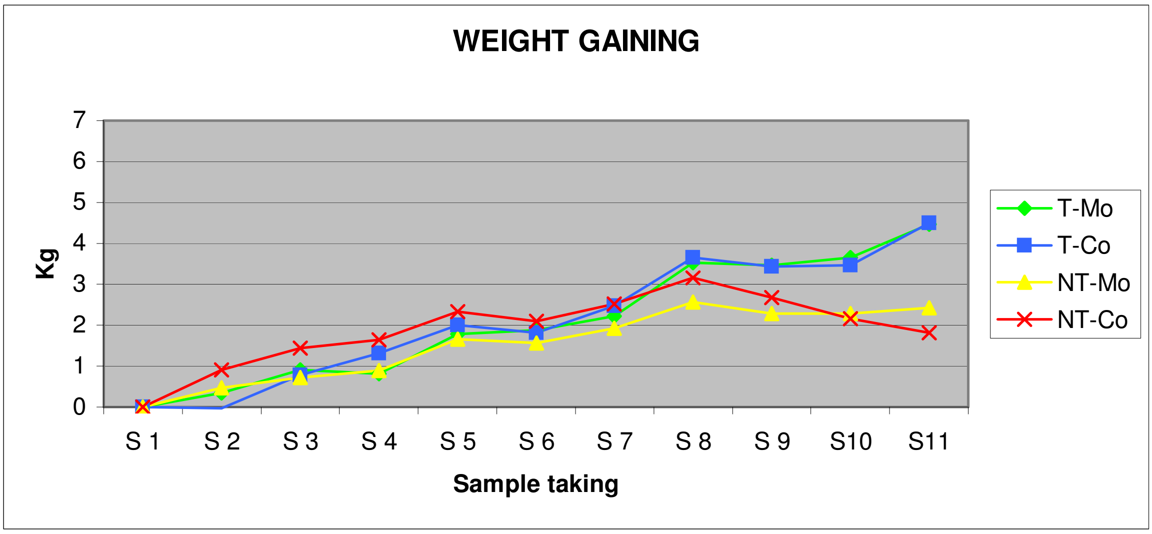

Figure 1. Weight gaining.

5.2 Hematocrit

There was no significant difference between NT-Co and NT-Mo (P>0,05) regarding the hematocrit, thus suggesting that resilience against GIN was similar in both groups. Both groups had a lower hematocrit level than the treated groups but remained within normal values.

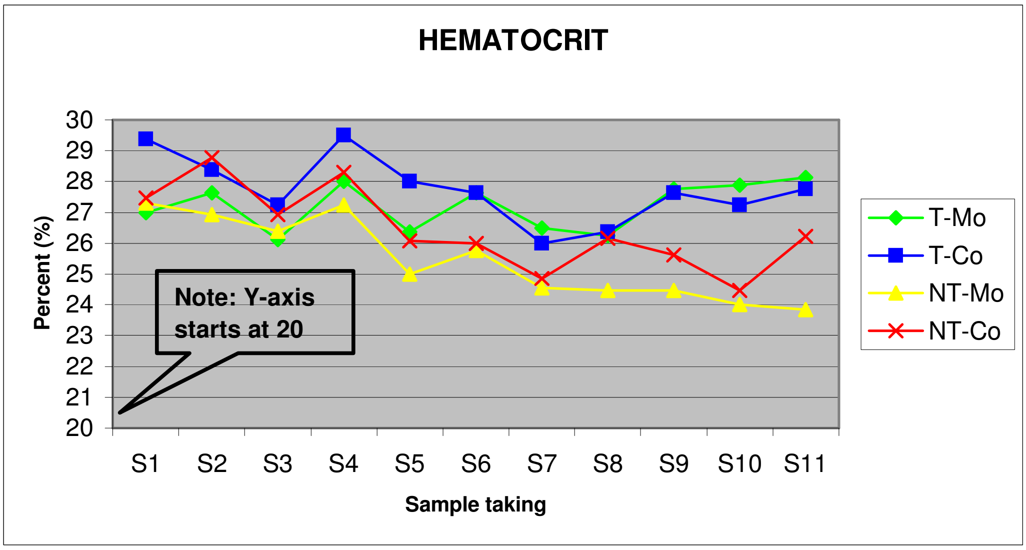

Figure 2. Hematocrit.

5.3 Faecal egg counts.

NT-Mo had a much higher level of worm eggs in their faeces than NT-Co ($P<0.0001$). However, the higher egg counts in the NT-Mo group was not associated with a reduced growth rate, or hematocrit level compared to NT-Co. These results showed that feeding molasses to the goats can improve their resilience against GIN but maize supplementation seems also to improve their resistance. This is the second trial where maize seems to affect worm population and worm fecundity in goats (Gutierrez-Segura et al., 2003). It is an interesting finding and warrants further studies as the underlying mechanisms are not yet revealed.

There was no difference in the amount of parasites between T-Mo and T-Co. They carried no parasites because of efficient anthelmintic, which was used only for experimental purposes.

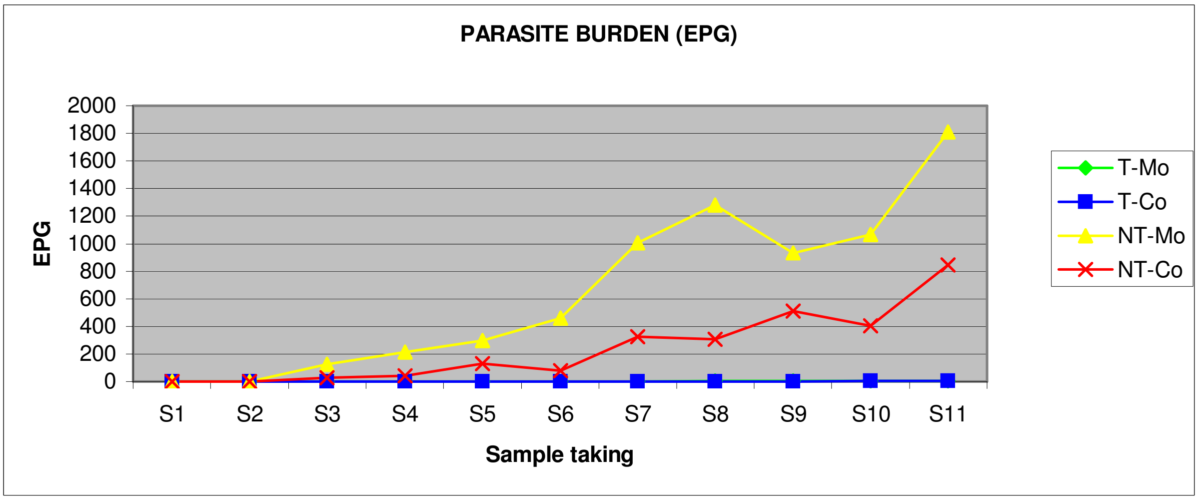

Figure 3. Parasite burden (EPG).

6. CONCLUSION

Supplementation with molasses improved the resilience of young goats to parasite infection, despite relatively heavy parasite burdens. Molasses therefore proves to be an efficient and relatively cheap form of supplementation for goats during the wet season in Mexico. It is a good alternative to maize, because of its affordability and that it has little value in nutrition for humans.

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All photographs taken by the author.

APPENDICES

A1. Methodology

A1.1 Keeping the experimental model kids nematode free

The goats were dewormed with effective anthelmintics two weeks before giving birth to the kids. They were dewormed a second time three days after giving birth. The kids stayed with their mothers the first few days, and were allowed to suckle two times a day the rest of their first month. They were completely separated from their mothers the second month and were only allowed to suckle when the goats were gathered to be milked each morning. The kids were not allowed to graze before the investigation but where fed non-infected feed in their pens.

A1.2 The McMaster technique

2 grams of faeces from each animal were collected and mixed with 28 grams of saturated sodium solution with a specific gravity of 1,240. A sample was collected with a 1 ml plastic syringue and a McMaster slide was filled with the liquid. The sample was read five minutes later with a 10 X microscope. The eggs found within the marked square of the chamber were counted and the number was multiplied with fifty to achieve the total amount of eggs per gram faeces (EPG).

A1.3 The microhematocrite capillary technique

Microhematocrite capillary tubes were filled with blood to ¾ of their length. The capillary tube end was blocked with clay and centrifuged in a hematocrite centrifuge with 14 000 revs per minute during 10 minutes. The hematocrite was then read.

Picture 4. A goat on the run.