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REGULAR ARTICLES

# Effect of gastrointestinal nematodes on serum copper and phosphorus of growing beef calves in northwestern Argentina

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**Abstract**
The aim of this work was to study the effect of gastrointestinal nematodes (GINs) on copper (Cu) and phosphorus (P) in blood of beef cattle in two ranches (R1 and R2) located in northwestern Argentina. In 2015–2016 (R1) and 2016–2017 (R2), in each ranch, 22 weaned female calves were divided into two groups: calves treated systematically with 200 mcg/kg moxidectin every 45–50 days (TG) and untreated calves (UTG). The following parameters were measured: number of fecal eggs (epg), fecal cultures, serum Cu and P levels, and live weight gain (LWG). Differences between groups were compared using analysis of variance and Tukey test. GIN infections in both ranches were subclinical and moderate, showing the highest epg (R1 = 907 ± 754; R2 = 1049 ± 1040) by mid-winter. Epg values of TG groups were always negligible (> 93% of moxidectin efficacy). The dominant nematode genera were *Cooperia* and *Haemonchus*. The average serum Cu values (μg/dl) indicated low (R1 = 49.7 ± 18) and severe (R2 = 27.2 ± 14) deficiency. The effect of treatments was evident in both ranches from late winter, with TG showing significantly ($p < 0.01$) higher serum levels in winter, spring, and early autumn (R1 = 65.1, 50.9, and 60.3; R2 = 48.0, 25.7, and 22.4) than UTG (R1 = 44.3, 33.9, and 32.9; R2 = 25.5, 18.2, and 16.4). There were no differences in serum P levels between groups. LWG of TG increased significantly ($p < 0.008$) (27.2% in R1 and 38.6% in R2), with respect to those of UTG. This study showed a negative effect of GIN on serum Cu values in moderately infected growing calves.

**Keywords** Cattle · Gastrointestinal nematode · Serum copper · Serum phosphorus · Effect

## Introduction

The competition for land resources between cattle and high-priced grain and oilseed crops in the central region in Argentina in the last 20 years has resulted in changes in beef production; cow-calf stocker operations were displaced towards marginal regions and rangelands of the north of the country. This new beef cattle scenario forced producers to adapt their production models to a subtropical climate; likewise, for systems to be competitive, they had to be intensified, which involved changes in the utilization of tropical seeded pastures and grain supplementation (Rearte 2010).

Cow-calf operations in these subtropical areas with almost no history of commercial intensive beef cattle raising have posed some competitive-limiting factors and health constraints, such as diseases affecting production (e.g., infection with gastrointestinal nematodes (GINs) and mineral deficiencies). These often subtle and subclinical infections can have important effects on the economic profitability of ranches. GIN infections are a significant constraint on the efficient raising of young cattle on pastures; indeed, it has been frequently reported that anthelmintic treatments of pastured cattle improve growth performance and general sanitary status in young bovines (Hawkins 1993; Suarez et al. 1999; Suarez et al. 2013; Borges et al. 2013; Charlier et al. 2014). Macroelement and microelement deficiencies contribute to metabolic disorders in the herd, affect immune response, and impair growth and development of young animals. Copper is an essential trace element for body and bone growth, pigmentation, and white blood cell function, and its deficiency severely reduces productivity (Hucker and Young

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1986; Radwińska and Żarczyńska 2014). Recent studies have also shown the economic impact of GIN infection in northern Argentina (Suarez et al. 2017, 2018a) as well as of moderate copper (Cu) and phosphorus (P) deficiency in cattle blood (Micheloud et al. 2017).

It has been suggested that ruminant parasites interfere with the absorption of Cu and P from the gastrointestinal tract (Bremner 1959; Bown et al. 1989; Poppi et al. 1990). The interaction of Cu and P with endoparasitism has been frequently reported (Hucker and Young 1986; Adogwa et al. 2005; Louvandini et al. 2009); however, this phenomenon has not been fully studied, especially in cattle raised in tropical regions.

The aim of this study was to contribute to the knowledge about the effect of GIN infection on serum Cu and P in growing replacement beef heifers with Cu or P deficiencies in the subtropical northwestern region of Argentina.

## Materials and methods

### Study area and animals

The study was conducted in two ranches, R1 and R2, located in the north and east of Salta province, respectively. Both regions are characterized by a subtropical climate with a dry winter season and summer rainfalls; R1 is located in an agro-ecological tropical area of piedmont forests and grasslands (− 24°29′54″ S and − 63°38′59″ W) and R2 in the semiarid Chaco region (− 22°49′42″ S and − 63°57′59″ W). These agro-ecological regions have a very irregular seasonal rainfall regime, with an annual mean of 900 and 580 mm for R1 and R2, respectively.

The study groups comprised 5-month-old red Brangus cross calves; in R1, calves were weaned early (at 3 months of age), whereas in R2, calves were in lactation at the beginning of the trial and were then weaned in April. Management was based on grazing of implanted megathermic pastures *Brachiaria brizantha* and *Magathyrsus maximum* in R1 and only *M. maximum* in R2, supplemented with ground corn during winter in both ranches.

### Experimental design

From March 2015 to March 2016 (R1) and from March 2016 to April 2017 (R2), 22 calves per ranch naturally infected with GINs were monitored. Two groups of 12 weaned female calves were formed to compare their responses to anthelmintic treatment: an untreated group (UTG) and a group treated systematically every 45–55 days (TG) with subcutaneous moxidectin (MXD, 200 mcg/kg). TG was included in the study with the sole purpose of obtaining an optimal unparasitized group that could be analyzed in terms of nematode effect; it was not used as a control group. The groups grazed together along with the entire stock of about more than 250 replacement heifers.

### Serum and parasitological analyses

Blood samples were collected by jugular venipuncture for serum extraction in March, June, September, and December and in March (R1) and April (R2) of the next year. Serum Cu levels were measured with an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200) and serum P levels by UV-visible spectrophotometry (Perkin Elmer Lambda 25). The reference normal values for serum Cu range from 65 to 150 μg/dl and for P, from 4 to 6 mg/dl (Underwood and Suttle 2003).

Fecal samples were collected every 45–50 days from the start of the study to September (R1) and February (R2); then, due to rainfalls, sampling intervals were extended, with the following sampling date being in December (both ranches) and the last one in March (R1) and April (R2). Individual fecal nematode egg counts (epg) were determined using the modified McMaster technique (Roberts and O'Sullivan 1949), where one egg represents 10 epg. Fecal cultures were performed in each group to assess the generic composition of nematode populations, according to Suarez (1997), and nematode larvae were identified using the morphological characters described by Van Wyk et al. (2004).

### Production assessment

Live weight gain (LWG) was assessed every 45–50 days by weighing female calves after 18 h of fasting.

### Data analysis

Least squares means analysis of variance was performed using InfoStat Statistical Software (Di Rienzo et al. 2008). Differences in LWG were compared via Tukey test.

## Results

### Parasitological analyses

Epg variation in the two studied ranches over the study period is shown in Figs. 1 and 2. At the start of the study, UTG mean epg values were moderate (595 ± 480) in R1 and low (102 ± 77) in R2; then, epg rose, peaking in mid-winter (R1, 907 ± 754; R2, 1049 ± 1040). From July to the end of observations, the two groups exhibited low epg, except for one peak (667 ± 928) in the middle of spring (667 ± 928) in R2. In TG groups of both ranches, epg values were very low during the period when the interval between treatments was shorter than 50 days

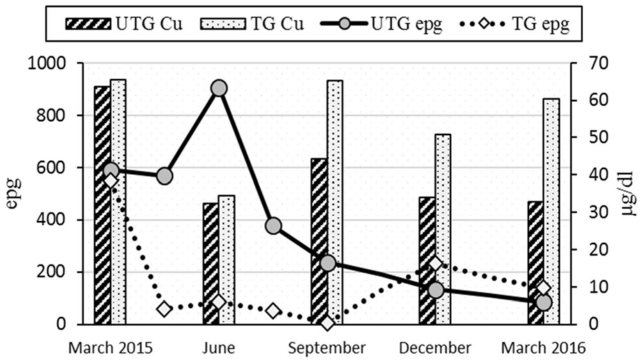


**Fig. 1** Mean of number of eggs per gram (epg) and serum copper levels of replacement heifer calves in R1 ranch during the study period. TG: systematically treated group; UTG: untreated group

(until the end of September in R1 and until February in R2), indicating a good MXD efficacy (93.5–95%) (Fig. 2).

In UTG, the dominant GIN genera recovered from fecal cultures were *Cooperia* (81.9%), followed by *Haemonchus* (15.5%) in R1 and *Haemonchus* (47.1%) and *Cooperia* (45.9%) in R2. Larvae of *Oesophagostomum* and *Trichostrongylus* were also recorded. The presence of the genera *Cooperia* and *Haemonchus* was observed mainly in autumn; in winter, only *Cooperia* remained until September, when *Haemonchus* was present again. In the TG, *Cooperia* was the only genus recovered during the periods when treatments were performed at 50-day intervals.

### Serum analyses

The results of serum Cu are presented in Figs. 1 and 2 and Table 1. In both ranches, average serum Cu levels were below the reference values. At the beginning of the trial, serum Cu levels were marginal in R1 and low in R2, and then fell in the UTG to values indicating minor and major deficiencies in R1 and R2, respectively. TG calves of both ranches showed significantly ($p > 0.02$) higher levels of serum Cu than UTG

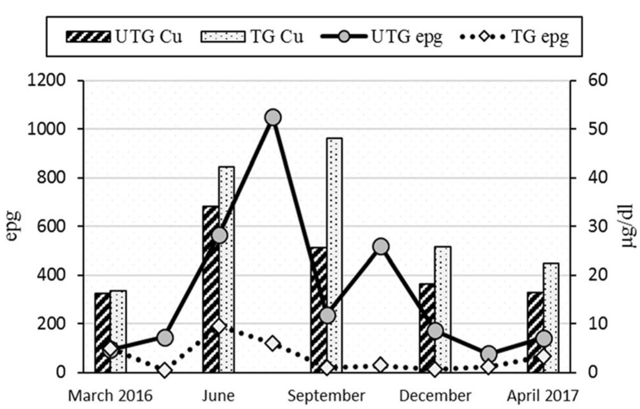


**Fig. 2** Mean number of eggs per gram (epg) and serum copper levels of replacement heifer calves in R2 ranch during the study period. TG: systematically treated group; UTG: untreated group

calves from the late winter samplings (September) to the end of the experiments.

The results of serum P are presented in Table 2. The average serum P values in R1 were within the ranges considered physiological until the end of winter; then, in December and March, serum P values decreased when the animals no longer received supplementation. In those months, average serum P values indicated phosphorus deficiencies. In R2 at the beginning of the experiment, before weaning, serum P values were subnormal; then, after weaning, from June to the end of the study, serum P values were within normal standards (Underwood and Suttle 2003). No significant differences were found in P values between groups in either ranch.

### Production data

Calves did not show clinical signs of verminous gastroenteritis, mineral deficiencies, or any other sign of health problems in the herd. By the end of winter, TG groups showed significant differences ($p < 0.001$, R1 and $p < 0.04$, R2) in LWG after treatment with respect to UTG groups. LWG of R1 calves was 51.3 kg (TG) and 30.8 kg (UTG) and of R2 calves, 45.3 kg (TG) and 25.3 kg (UTG). Then, differences in LWG between groups continued to increase until the end of the trial, when total LWG remained significantly ($p < 0.008$) higher in TG (R1, 131.9 kg; R2, 146.5 kg) than that in UTG (R1, 103.7 kg; R2,105.2 kg). LWG of TG increased by 27.2% (R1) and 38.6% (R2) with respect to those of UTG.

## Discussion

As previously assessed by egp count (Suarez et al. 2018a; Suarez et al. 2018b), parasitic burdens increase towards late autumn or early winter, damaging the LWG of the calves. In the present work, in both ranches, significant differences in Cu levels in response to anthelminthic treatments were first detected towards the end of winter (September sampling). The lack of differences in Cu levels in the June samplings may be attributed to the fact that the liver reserves still had copper. However, in the case of R2 calves that suffered a major Cu deficiency, Cu levels tended to show some differences in the second sampling, being higher in the TG than in the UTG (Fig. 2). The studies of relationships between concurrent Cu deficiency and GIN infection in ruminants are very scarce. Gastrointestinal nematodiasis can significantly exacerbate an existing Cu deficiency in sheep (Hucker and Young 1986); in calves heavily infested with *Haemonchus*, *Bunostomum*, *Oesophagostomum*, and *Cooperia* and with a consequent severe anemia and hypoproteinemia, Bremner (1959) evidenced depressed liver and blood copper levels, whereas in worm-free controls, those values remained normal. In this field experiment, GIN genera recovered from fecal cultures were the same

**Table 1** Serum copper levels (µg/dl) of the untreated group (UTG) and anthelmintic-treated group (TG) in the two ranches during the experiment

| Groups | March | June | September | December | March/April |
|---|---|---|---|---|---|
| Ranch 1 (March 2015–March 2016) | | | | | |
| UTG Cu | 65.4 ± 16.9 a | 32.3 ± 16.5 a | 44.3 ± 10.6 a | 33.9 ± 11.2 a | 32.9 ± 16.3 a |
| TG Cu | 63.7 ± 12.0 a | 34.5 ± 10.0 a | 65.2 ± 13.7 b | 50.9 ± 11.2 b | 60.3 ± 8.4 b |
| Ranch 2 (March 2016–April 2017) | | | | | |
| UTG Cu | 16.2 ± 5.7 a | 34.0 ± 12.8 a | 25.5 ± 9.2 a | 18.2 ± 7.3 a | 16.4 ± 4.0 a |
| TG Cu | 16.8 ± 5.2 a | 42.3 ± 10.8 a | 48.0 ± 13.3 b | 25.7 ± 11.3 b | 22.4 ± 6.1 b |

Different letters indicate significant differences ($p < 0.05$)

as those described by Bremner, but in different proportions: in R1, more than 90% of GINs recovered were *Cooperia*, especially *C. punctata* (Suarez et al. 2018a) and in R2, *Cooperia* and *Haemonchus* genera were dominant.

Copper is found in many enzymes and plays a very important regulatory role in biochemical processes; this element is particularly important in growing animals, since its deficiency causes a great variety of symptoms (Gooneratne et al. 1989). Primary deficiency is observed in regions where copper content of soil or plants is low or secondary due to high concentrations of copper antagonists, such as molybdenum, sulfur, and iron. In the two studied ecoregions, copper deficiency is probably primary; however, studies on soil or pasture Cu content are required to confirm deficiency.

The seasonal variation of copper in R1 shows that serum Cu levels in calves are marginal at weaning and then show an evident deficiency. The highest levels of copper ($p < 0.0001$) at weaning are likely due to an increase in Cu accumulation during fetal development; moreover, significantly higher Cu concentrations in liver were observed in the fetus than in the mothers (Gooneratne and Christensen 1989). Conversely, the lowest Cu values in R2 calves were recorded at the beginning of the experiment, when they were still lactating; then Cu values rose in June and September, when calves were supplemented with corn grain, and went down again in December and April, when animals were fed only on pastures.

Interestingly, there were also differences ($p < 0.013$) in Cu levels between UTG and TG of R2 at the end of the trial (Table 1), even when treatments were made at a long interval (no treatment between February and late April, i.e., 70 days) and the probable reinfection of TG, once the effect of MXD on GIN was lost (Eysker et al. 1996). These differences may be attributed to Cu liver reserves. Likewise, the Cu levels of both groups were very low and similar to those obtained at the beginning of the trial, which may be due to Cu deficiency in soils and pasture.

Until mid-winter, significant production differences were recorded as a response to anthelmintic treatments in both ranches when GIN infections co-occurred with significantly lower Cu levels in UTG than in TG. The responses obtained in R1 were due to an infection caused by *Cooperia punctata* and *C. pectinata* (Suarez et al. 2018a), which cause anorexia, thickening of intestinal villi and excessive amount of mucus, gut loss of plasma proteins, and growth delay (Stromberg et al. 2012). In R2, the dominant presence of *Haemonchus* together with *Cooperia* may aggravate the effects of parasitism, as confirmed by Vieira-Bressan et al. (1995) in studies in Brazil on calves concurrently infected with *C. punctata* and *Haemonchus placei*. Similar responses of LWG and Cu levels to treatments were reported in drenched vs untreated calf groups in the central region of Argentina, which were attributed to the effect of mixed parasite infections with *Ostertagia ostertagi*, *Trichostrongylus axei*, *Cooperia oncophora*, and *Haemonchus placei* (Suarez et al. 1990).

In ruminants, Cu is absorbed partially in forestomachs and mainly in the stomach and the small intestine, where copper ions are bound by low-molecular-weight proteins (Underwood and Suttle 2003). By far, one of the most

**Table 2** Serum phosphorus levels (mg/dl) of the untreated group (UTG) and anthelmintic-treated group (TG) in the two ranches during the experiment

| Groups | March | June | September | December | March/April |
|---|---|---|---|---|---|
| Ranch 1 (March 2015–March 2016) | | | | | |
| UTG P | 6.81 ± 1.2 a | 5.51 ± 0.1 a | 6.35 ± 1.2 a | 3.29 ± 0.4 a | 3.56 ± 0.4 a |
| TG P | 6.88 ± 1.2 a | 5.29 ± 0.5 a | 6.44 ± 1.0 a | 3.06 ± 0.5 a | 3.57 ± 0.3 a |
| Ranch 2 (March 2016–April 2017) | | | | | |
| UTG P | 2.53 ± 0.4 a | 5.06 ± 0.1 a | 5.75 ± 0.7 a | 4.88 ± 0.9 a | 4.07 ± 0.7 a |
| TG P | 2.47 ± 0.3 a | 5.15 ± 0.4 a | 5.73 ± 0.6 a | 5.00 ± 0.5 a | 4.61 ± 0.4 a |

Different letters indicate significant differences ($p < 0.05$)

significant effects of parasitism on the host is a depression in food intake, and this could surely affect the source of Cu income (Sykes et al. 1988; Fox 1997). On the other hand, during mixed infections, abomasal and intestinal nematodes were thought to affect copper metabolism by interfering with copper absorption from the gastrointestinal tract, increasing the pH of the gastric environment and damaging the intestine (Poppi et al. 1990; Holmes 1993). Bang et al. (1990) observed that elevated abomasal pH was associated with reduced net uptake of Cu in the liver of lambs infected with *Teladorsagia circumcincta*. Likewise, GINs produce plasma losses into the gut (Bown et al. 1991; Coop and Holmes 1996). In summary, it is probable that the descent in Cu status was due to the decreased uptake of dietary Cu, the depression of Cu absorption, and the loss of Cu with plasma into the gut.

No differences were found in serum P levels, even on the sampling dates when there were deficient levels of P of growing calves naturally infected with GINs. On the contrary, sheep experimentally infected with *Trichostrongylus* and *Teladorsagia* parasites showed depressed plasma Cu and P concentrations associated with reduction in absorption and endogenous Cu and P losses (Bown et al. 1989). Accordingly, there is good evidence for reduced P absorption in intestinal sheep parasitism (Wilson and Field 1983; Poppi et al. 1985). In Brazil, Louvandini et al. (2009) showed that P metabolism in experimentally infected calves subjected to a trickle experimental infection with *C. punctata* negatively affected P kinetics, as a consequence of lower dry matter and P intake, as well as lower P absorption and retention. These results show subnormal P values in both ranches when the animals were managed exclusively on pastures without supplementation. Because the deficit of P is mainly a primary deficiency (Goff 2009), it can be assumed that pastures would have low concentrations.

## Conclusion

The study shows that with subclinical infections with the genus *Cooperia* in both ranches, concurrently with *Haemonchus* in R2, a negative effect of GINs on serum copper values in growing calves was observed under the conditions of the beef production systems of northwestern Argentina region. In both ranches, differences in LWG between groups in response to treatments showed the effect of GINs, and this production decrease might have been enhanced by the existing Cu deficiency. Finally, these findings demonstrated that gastrointestinal nematodiasis significantly aggravated an existing Cu deficiency and reinforce the need for effective GIN control in field situations where Cu deficiency and nematodiasis co-occur.

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## Compliance with ethical standards

The procedures applied have been approved by the Ethical Review Committee (CICUAL: Institutional Committee of Care and Use of Experimental Animals) of the Regional Center of Salta-Jujuy of the National Institute of Agricultural Technology (INTA) under the approval act: N°2/18.

**Conflict of interest statement** The authors declare that they have no conflict of interests.

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