

SERUM PEPSINOGEN CONCENTRATION IN SUFFOLK AND POLWARTH EWES AT THE END OF GESTATION, DURING LACTATION AND AFTER WEANING

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SUMMARY: The relationship between eggs per gram of feces (EPG) and serum pepsinogen level was studied in Polwarth and Suffolk ovine breeds. They were naturally infected by gastrointestinal nematodes and were studied at the end of gestation, during lactation and after weaning. In this study, twenty sheep (10 Suffolk and 10 Polwarth) between three and four years old, raised together paddocks on a rotational system, were used. All sheep were treated with Closantel Ó (10 mg/kg) thirty days before parturition. The serum pepsinogen and the EPG were determined in the second and first week before parturition as well as in the second, fourth and sixth week of lactation and in the first and second week after weaning. The highest percentages of larval in the fecal cultures were *Haemonchus*. A periparturient rise in EPG were observed in both breeds, Suffolk and Polwarth. The EPG had a huge increase near parturition and kept high during lactation, however, without a significant increase of the serum pepsinogen level. After weaning, the EPG showed a continuous decrease associated with an increase in the serum pepsinogen. There was no significant correlation ($p > 0,05$) between the EPG and the serum pepsinogen in Suffolk breed, whereas there was a strong negative correlation ($r = -0,76$; $p < 0,0001$) in Polwarth sheep, but only after weaning. The results suggest that the increase in serum pepsinogen, after weaning, is related with a more efficient immune response of the hosts against the nematode infection, after the end of the periparturient relaxation of resistance.

KEY WORDS: Pepsinogen, Nematoda, *Haemonchus*, Sheep, peri-parturient rise.

INTRODUCTION

Close to parturition, pregnant ewes present an increase in worm burden and high eggs per gram (epg) counts, which persist during lactation and decrease after weaning. This phenomenon was denominated in Brazil, by AMARANTE *et alii* (1992), as the periparturient phenomenon (FP), and is known in other countries as "post-parturient rise", "lactation rise" and "spring rise". The mechanisms by which the FP occurs are yet unknown, although it is believed that the FP is caused by an unspecific immunosuppression of endocrine origin, due to the hormonal alterations that occur close to parturition and during lactation (SOULSBY, 1987). The FP varies in intensity according to the resistance of the sheep breed to helminthiasis (COURTNEY *et alii*, 1984; AMARANTE *et alii*, 1992).

The determination of the blood pepsinogen concentration has been recommended as an auxiliary technique for the

diagnosis of ruminant parasitic gastroenteritis since it was employed for the first time by ANDERSON *et alii* (1965). The mechanism by which the blood pepsinogen concentration increases is still unknown, but may be due to at least 3 factors: (1) decreased conversion of pepsinogen into pepsin due to alcalinization of the abomasal contents after infection (JENNINGS *et alii*, 1966); (2) increase in abomasal permeability to macromolecules such as pepsinogen (MURRAY, 1969); (3) increase in the production of pepsinogen as a direct result of the secretion of excretory/secretory products by the parasites (McKELLAR *et alii*, 1990).

There is evidence that the increase in blood pepsinogen in adult ewes grazing pastures contaminated with nematode infective larvae is due to a hypersensitivity immune response that increases the permeability of the abomasal mucosa (YAKOOB *et alii*, 1983b). According to STEAR *et alii* (1995), the blood pepsinogen concentration reflects the host response

to nematode infection, while the epg counts reflect the behaviour of the parasite inside the host. According to these authors, the concomitant determination of epg counts and pepsinogen makes the identification of nematode resistant and susceptible ewes more effective.

Most studies concerning blood pepsinogen concentration were carried out in ewes experimentally infected with nematodes (COOP, 1971; YAKOUB *et alii*, 1983b, FOX *et alii* 1988, MOSTOFA *et alii*, 1990; LAWTON *et alii*, 1995), while studies using naturally infected ewes are scarce (THOMAS & WALLIER, 1975; YAKOUB *et alii*, 1983a), specially during the periparturient period, when the immunosuppression responsible for the FP takes place.

Aiming to contribute for a better understanding of the FP, the variation in epg counts and serum pepsinogen concentration were studied comparatively in Suffolk and Polwarth ewes naturally infected by nematodes, at the end of gestation, during lactation and after weaning.

MATERIALS AND METHODS

Ten Suffolk and ten Polwarth ewes, all between three and four years old, belonging to the Experimental Goat and Sheep Station (PECO) of the Instituto de Zootecnia in Itapetininga – SP. The 20 ewes were raised together, in a rotational grazing system with a mean of 10 animals/ha. At night the animals were brought to a night shelter (150 m²) where they were fed concentrate with 9-12% CP (150 g/head/day), corn silage, Pennisetum grass chaff and Bermuda grass hay (4 kg/head/day) and mineral supplement ad libitum. Thirty days before parturition, all ewes were treated with Closantel* (10 mg/kg bodyweight) and triple vaccine** (carbuncle, gas gangrene and pulpy kidney). As recommended by SIQUEIRA (1996), early weaning took place six weeks after parturition.

Seven fecal and blood samples were collected from all 20 ewes and grouped into seven experimental moments: second and first weeks pre-lambing (2PP e 1PP); second, fourth and sixth weeks of lactation (2LA, 4LA e 6LA); first and second weeks after weaning (1PD e 2PD).

In all fecal samples, obtained directly from the rectal ampulla, the determination of the gastrointestinal nematode egg counts per gram feces (epg) was performed by the modified technique of GORDON & WHITLOCK (1939). Coprocultures were made according to the method of ROBERTS & O'SULLIVAN (1950), from a mixture of the fecal samples of each breed, to obtain infective larvae, which were identified according to KEITH (1953).

The blood for serum pepsinogen determination was obtained by jugular venipuncture and kept at room temperature for coagulation and retraction of the clot. The obtained sera were kept at -20°C until processing at the laboratory. The serum

pepsinogen concentration was determined by the method of MIRSKY (1952), using sheep hemoglobin as substrate and the results were expressed as mU tyrosine. The sheep hemoglobin substrate was obtained by the extraction and purification method described by HENRY *et alii* (1974).

According to the recommendations of SIEGEL (1956), the values of the epg counts and serum pepsinogen of the two breeds studied were compared in the various experimental moments by the Mann-Whitney U test and between the various moments for each breed by the test of Friedman (SIEGEL, 1956). The correlation between the epg counts and serum pepsinogen in the preparturient, lactation and post-weaning periods were estimated by the calculation of the Spearman coefficient (SIEGEL, 1956).

RESULTS AND DISCUSSION

The results of coprocultures revealed the genus *Haemonchus* predominated in both breeds and represented an average of 89,73% of the identified larvae, during the whole experimental period. *Trichostrongylus* was the second most prevalent genus, with an average of 8,24 of the identified larvae. In some moments, the coproculture was positive for the genera *Oesophagostomum* and *Cooperia*, representing an average of 1,88% and 0,26%, respectively, of the identified larvae during the different moments of the present study.

Table 1 shows that in both breeds, Suffolk and Polwarth, the epg counts rose sharply after the first week preparturition (1PP), reaching maximum values on the fourth week of lactation (4LA). After the fourth week of lactation (4LA), for both breeds, the epg values decreased, and the epg mean of the Suffolk ewes on the second week after weaning (2PD) were lower than that of the second week preparturition (2PP). For Polwarth ewes, on the other hand, the mean epg values up to the second week after weaning (2PD) remained higher than that for the preparturition period (1PP e 2PP). These results of increased epg at the end of gestation and during lactation, and a decrease after lamb weaning are characteristic of the FP, as described by AMARANTE *et alii* (1992). For Suffolk ewes (Table 1), the values of epg counts of the second week preparturition (2PP), first and second weeks post-weaning (1PPD e 2PD) were significantly lower ($p < 0,05$) than that of the fourth week of lactation (4LA), while the values of the second week after weaning (2PD) were significantly lower ($p < 0,05$) than those of the second week of lactation (2LA). For Polwarth ewes, however, the values of the epg counts (Table 1) of the fourth and sixth weeks of lactation (4LA e 6 LA) and of the first week after weaning (1PD) were significantly higher ($p < 0,05$) than those of the second week preparturition (2PP).

Although in all moments the mean epg counts of Polwarth ewes had been higher than those of the Suffolks (Table 1), this

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Table 1 - Means and standard deviations of the counts of eggs per gram feces (epg) and of the serum pepsinogen concentration (mU tyrosine) of Suffolk and Polwarth ewes in various weeks during preparturition (PP), lactation (LA) and post-weaning (PD).

WEEK	EPG SUFFOLK	EPG POLWARTH	PEPSINOGEN SUFFOLK	PEPSINOGEN POLWARTH
2PP	1370 ± 1857 ^{Ab*}	1640 ± 1851,24 ^{Aa}	420,74 ± 217,19 ^{Aab}	328,89 ± 174,70 ^{Aa}
1PP	2760 ± 2983 ^{Aabc}	5600 ± 5366,18 ^{Aab}	355,56 ± 254,12 ^{Aa}	320,00 ± 140,23 ^{Aa}
2LA	4570 ± 3305,56 ^{Aab}	7260 ± 7450,01 ^{Aab}	533,33 ± 533,33 ^{Aab}	322,96 ± 227,14 ^{Ba}
4LA	6670 ± 4553,39 ^{Aa}	11450 ± 9599,68 ^{Ab}	444,45 ± 226,51 ^{Aab}	311,11 ± 158,79 ^{Aa}
6LA	4690 ± 5567,05 ^{Aabc}	9990 ± 8099,58 ^{Ab}	681,49 ± 237,04 ^{Ab}	367,41 ± 177,34 ^{Ba}
1PD	1900 ± 1986,61 ^{Ab}	7170 ± 5311,42 ^{Bb}	684,45 ± 128,35 ^{Ab}	328,89 ± 160,14 ^{Ba}
2PD	550 ± 704,35 ^{Abc}	5650 ± 7269,60 ^{Bab}	565,93 ± 284,69 ^{Aab}	565,93 ± 442,23 ^{Aa}

* Different lower case letters in the same line represent significant difference (p<0,05) between breeds. Different lower case letters in the same column represent significant differences (p < 0,05) between moments

Table 2 - Results of the coefficients of correlations posts?? (r) estimated by the method of Spearman and their respective p-values relative to the counts of eggs per gram faeces (epg) and the serum pepsinogen concentration of Suffolk and Polwarth during the preparturition, lactation and post-weaning periods.

Moments	Suffolk	Ideal
Preparturition	r = 0,24 (p = 0,29)	r = -0,26 (p = 0,25)
Lactation	r = -0,26 (p = 0,16)	r = -0,38 (p = 0,03)
Post-weaning	r = -0,22 (p = 0,33)	r = -0,76 (p = 0,0001)

difference was statistically significant (p < 0,05) on the two weeks post-weaning (1PD e 2PD) only. These results demonstrate that the periparturient rise (FP) occurred in both studied breeds with similar intensity, however, in Suffolk ewes the worm burden estimated by epg counts decreased faster than in the Polwarths.

Considering that studies have demonstrated that there is a direct correlation between epg counts and pasture contamination with infective larvae (L₃) of gastrointestinal nematodes (WOOLASTON, 1992), these results suggest that the Suffolk breed is more resistant than the Polwarth.

According to Table 1, in Suffolk ewes the serum pepsinogen concentration in the sixth week of lactation (6LA) and in the first week after weaning (1PD) were significantly (p < 0,05) higher than that of the of the first week preparturition (1PP). On the other hand, for Polwarth ewes the serum pepsinogen concentration in the various moments did not differ significantly (p > 0,05). Suffolk ewes had significantly higher serum pepsinogen levels (p < 0,05) than the Polwarths, in the second and sixth weeks of lactation (2LA e 6 LA) and in the first week after weaning (1PD).

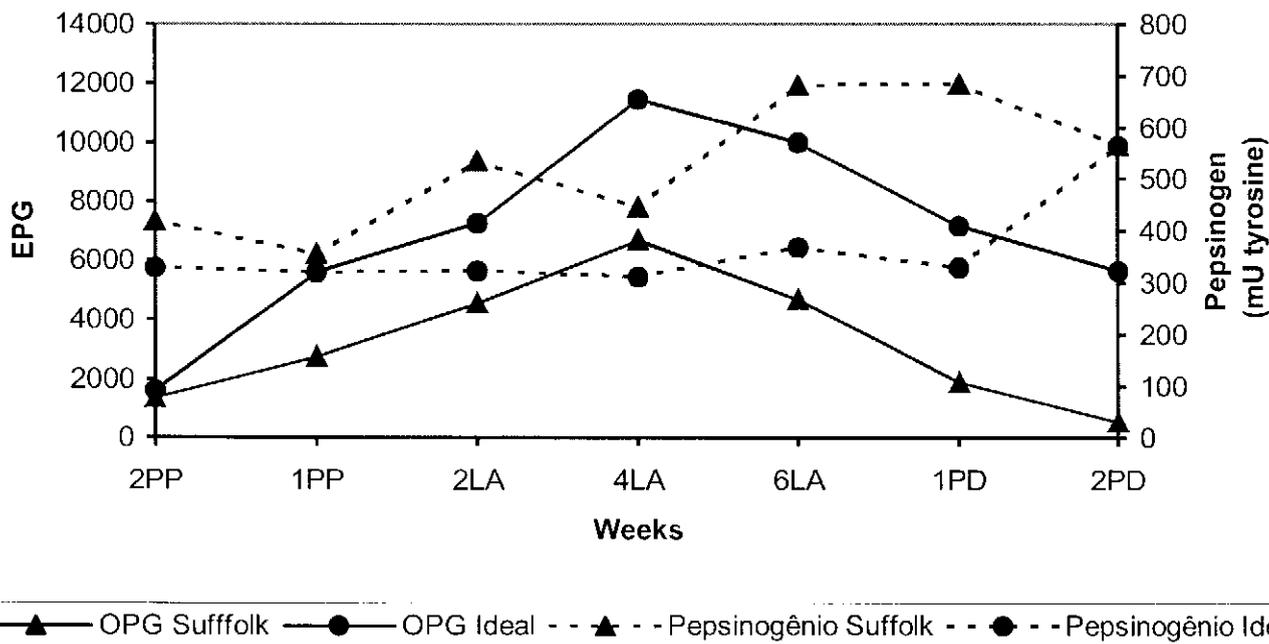


Figure 1 - Mean counts of eggs per gram faeces (epg) and serum pepsinogen concentration (mU tyrosine) of Suffolk and Polwarth ewes in various weeks preparturition (PP), lactation (LA) and post-weaning (PD).

The graph in Figure 1 reveals that in all moments the serum pepsinogen concentration showed large variation and that its increase is more closely associated with a decrease in epg counts from the sixth week of lactation (6LA) onwards, than with the increase in epg counts observed between the first week preparturition (1PP) and the fourth week of lactation (4LA). These results suggest that the serum pepsinogen concentration seems of little value for the diagnosis of gastrointestinal parasitism, naturally acquired by adult grazing ewes. MYLREA & HOTSON (1969) also observed extensive variation in the blood pepsinogen concentration in grazing cattle and concluded that the determination of serum pepsinogen has little value as an auxiliary diagnostic technique for cattle gastrointestinal parasitoses. COOP (1971) and more recently LAWTON *et alii* (1996), observed that many ewes naturally infected by nematodes did not present increased plasma pepsinogen concentration.

The values in Table 2 reveal that the correlation between the epg counts and the serum pepsinogen concentration was usually negative and significant only during lactation ($r = -0,38$; $p = 0,03$) and after weaning ($r = -0,76$; $p = 0,0001$) for Polwarth ewes. These results agree with those of other studies, which have demonstrated that the correlation between the blood pepsinogen concentration and the epg counts is negative (THOMAS & WALLER, 1975) or non-existent (STEAR *et alii*, 1995).

The results shown in Table 1 and in Figure 1 clearly demonstrated that the sharp increase in epg counts that occurred between the second week preparturition (2PP) and the fourth week of lactation (4LA) was not accompanied by a significant increase in serum pepsinogen. Considering that during this period ewes are unspecifically immunosuppressed, what increases their susceptibility to nematodes (SOULSBY, 1987); these results suggest that the decrease in the immune response, associated with the FP, might have inhibited the hypersensitivity reaction responsible for the increase of permeability of the gastric mucosa and subsequent increase in serum pepsinogen concentration, as observed in the present study. In this sense, YAKOUB *et alii* (1983b) reported that the increase in plasma pepsinogen concentration in adult ewes parasitized by gastrointestinal nematodes might be related to an immune reaction that increases the permeability of the gastric mucosa. Supporting this hypothesis, the results in Figure 1 reveal an increase in serum pepsinogen concentration in both breeds (Suffolk and Polwarth), which coincides with a decrease in epg counts at the end of lactation and after weaning. Then, the increase in the serum pepsinogen concentration, observed in this study, is probably related to an improved host immune response at the end of lactation and after weaning. STEAR *et alii* (1995) state that in adult ewes naturally infected by gastrointestinal nematodes, the increase in plasma pepsinogen concentration has no association to worm burden, but alternatively, reflects the host response to infection.

Other studies should be carried out aiming at a better understanding of the possible relationship between the ability of the host to mount an immune response and the serum pepsinogen concentration in ewes parasitized by gastrointestinal nematodes.

SUMÁRIO

Estudou-se comparativamente a variação na contagem de ovos por grama de fezes (OPG) e a concentração sérica de pepsinogênio em ovelhas das raças Suffolk e Ideal naturalmente infectadas por nematódeos gastrintestinais, ao final da gestação, durante a lactação e após o desmame. Para tal, utilizaram-se 10 ovelhas da raça Suffolk e 10 da raça Ideal, todas entre 3 e 4 anos de idade, criadas juntas em sistema rotacional de pastagem. Todas as ovelhas foram tratadas 30 dias antes do parto com Closantel (10 mg/kg). Determinou-se a concentração de pepsinogênio sérico e realizaram-se exames fecais na segunda e primeira semana pré-parto, na segunda, quarta e sexta semana de lactação e na primeira e segunda semana após o desmame dos cordeiros. Os resultados das coproculturas demonstraram que as ovelhas foram parasitadas predominantemente por nematódeos do gênero *Haemonchus* e que o fenômeno do periparto manifestou-se de forma similar em ambas as raças (Suffolk e Ideal), de modo que a contagem de OPG aumentou acentuadamente próximo ao parto e se manteve elevada durante a lactação, sem que ocorresse neste mesmo período um aumento significativo da concentração sérica de pepsinogênio. Após o desmame, a contagem de OPG apresentou um acentuado declínio associado a uma elevação da concentração sérica de pepsinogênio. Em ovelhas da raça Suffolk, não se verificou correlação significativa ($p > 0,05$) entre a contagem de OPG e a concentração sérica de pepsinogênio, enquanto que em ovelhas da raça Ideal, esta correlação, foi fortemente negativa ($r = -0,76$; $P < 0,0001$), mas apenas após o desmame. Os resultados sugerem que o aumento da concentração sérica de pepsinogênio, após o desmame, está relacionado a resposta imune mais eficiente dos hospedeiros contra a infecção por nematódeos, após o término da imunossupressão causada pelo fenômeno do periparto.

PALAVRAS CHAVE: pepsinogênio, nematódeos, *Haemonchus*, ovinos, fenômeno do periparto.

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