Accuracy of the FAMACHA© method in ewes fed different levels of crude protein

Francisco de Assis Fonseca de Macedo[1*], Fábio José Lourenço[2], Graziela Aparecida Santello[3], Elias Nunes Martins[1], Natália Holtz Alves Pedroso Mora[1] and Alexandre Agostinho Mexia[4]

[1]Universidade Estadual de Maringá, Avenida Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. [2]Universidade Federal do Mato Grosso, Cuiabá, Mato Grosso, Brazil. [3]Escola Superior Batista do Amazonas, Manaus, Amazonas, Brazil. [4]Universidade do Estado de Mato Grosso, Cáceres, Mato Grosso, Brazil. *Author for correspondence. E-mail: fafmacedo@uem.br

ABSTRACT. The accuracy of the FAMACHA© method was evaluated on the identification of female sheep fed two levels of crude protein, naturally infected with *Haemonchus contortus*, by means of the correspondent hematocrit value. Forty-seven female sheep of the breeds Santa Inês (n = 16), Texel (n = 16) and Ile de France (n = 15) aged between eight and twelve months were assigned to two treatments, 12 or 16 % crude protein in the diet. All the animals were wormed thirty days before the first data collections, which were done fortnightly between July 2005 and March 2006. The color of the ocular conjunctiva was individually evaluated according to the precepts of the FAMACHA© method and the hematocrit value of each animal was obtained in laboratory. A correlation of 1:0.7991 was found between the hematocrit values and the classification given by the FAMACHA© method aiming to identify animals with different degrees of anemia. The method was efficient to identify animals to worm, thus representing a support in the identification of animals susceptible to *Haemonchus contortus*.

Keywords: anemia, anthelmintic, nematode, parasitosis.

Acurácia do método FAMACHA© em fêmeas ovinas alimentadas com diferentes níveis de proteína bruta

RESUMO. Avaliou-se a precisão do método FAMACHA© na identificação de fêmeas ovinas alimentadas com dois níveis de proteína bruta, infectadas naturalmente por *Haemonchus contortus* por meio do valor de hematócrito correspondente. Foram utilizadas 47 fêmeas ovinas das raças Santa Inês (n = 16), Texel (n = 16) e Ile de France (n = 15), com idades entre oito e 12 meses. Dentro de cada raça, os animais foram distribuídos em dois tratamentos, 12 ou 16% de proteína bruta total na dieta. Todos os animais foram vermifugados 30 dias antes das primeiras coletas de dados que foram feitas quinzenalmente, no período entre julho de 2005 e março de 2006. Avaliou-se individualmente a mucosa ocular de todos os animais em concordância com o preceituado pelo método FAMACHA©, bem como se obteve o valor do hematócrito de cada animal avaliado em laboratório. Obteve-se uma correlação de 1:0,7991 dos valores de hematócrito e a classificação pelo método FAMACHA© visando a identificação de animais acometidos por diferentes graus de anemia. O método mostrou-se eficiente na identificação dos animais que necessitam de tratamento antiparasitário constituindo-se como suporte na identificação de animais susceptíveis ao *Haemonchus contortus*.

Palavras-chave: anemia, anti-helmíntico, nematódeo, parasitose.

Introduction

Infections caused by gastrointestinal parasites are a significant cause for economic losses in sheep. Worms represent the greatest and worst health problem concerning the sheep industry and can economically impede the sheep production (BUZZULINI et al., 2007), and may account for 60% of losses in the activity. This is due to an overspending with anthelmintics and the mortality of recently calved females and young animals, with consequent reduction in herd replacement and in reproductive indices of these animals (ABRÃO et al., 2010).

In Brazil, the control of helminth infections in livestock is costly in different seasons of the year. The tropical climate of this country has humid summer and mild winter, thus requiring farmers to worm more frequently sheep herds compared with the production under temperate climate. Temperature, radiation and rainfall influence the survival of larvae of *Haemonchus contortus* in the pasture (SOUZA et al., 2000). The animals are

infected with larvae of *Haemonchus* sp. while they are grazing and depending on many factors like breed, age and nutritional condition, the negative impact over the individual and herd productivity can be substantial.

Several strategies are studied in order to reduce animal infection and pasture contamination. However, for Van Wyk and Bath (2002), the application of individual techniques to identify animals with hematophagous parasite should be rapid, easy to use and low-cost. In this way, targeted selective treatments (TST) have been recently proposed, the best being the FAMACHA© method, developed in South Africa. The test is based on the principle that the color of mucous membranes is correlated with the degree of anemia status an animal (RILEY; VAN WYK, 2009), and so, it reflects the hematocrit.

The FAMACHA© card contains five classifications referent to the color of the ocular conjunctiva, from bright red (probably normal) to pale white (probably anemic), each one associated with a specific range for the hematocrit value, with a correlation of 0.8 and reliability above 95%. This card and methods of use were name FAMACHA©, in honor of one of authors. In relation to the use of antiparasitic drugs, Molento et al. (2004) found a reduction of dosages/costs of the order of 75.6%, with no deaths, when used the FAMACHA© method.

Protein is a structural component of the animal body. It composes muscles and tissues of many organs, including the gastrointestinal tract, liver, immune system and blood. Diets with high levels of protein provide enhanced immune response, especially to breeds naturally more resistant to hemonchosis (BRICARELLO et al., 2005). Regarding the diet, protein supplementation is an important factor to increase the resilience and resistance of sheep against simple or mixed infections caused by gastrointestinal nematodes (VAN HOUTERT; SYKES, 1996). Studies show the influence of protein supplementation in the diet for sheep infected with endoparasites, with an increase in resistance and degree of immunity (HAILE et al., 2002), since it enhances the production of antibodies. Veloso et al. (2006) also evidenced that, after the third month, the animals that received a high-protein diet showed lower values of eggs per gram (EPG) than the animals that had a diet with low protein levels.

Considering the impact of worms on Brazilian sheep husbandry, the aim of this study was to prove that the FAMACHA© method can be efficiently

used to control *Haemonchosis sp.* in young female sheep fed two levels of crude protein.

Material and methods

The experiment was carried out from July 2005 to December 2006, at the Centro de Pesquisa do Arenito of the Universidade Estadual de Maringá (UEM), in the municipality of Cidade Gaúcha, northwest Paraná State, located in the latitude 23° 25's and longitude 51° 55' W, at 554.9 m altitude. Solar radiation was obtained by a local micro-meteorological station, presenting average 848.65 w m^{-2} in the winter, 1,133.75 w m^{-2} in the spring, 1,197.83 w m^{-2} in the fall and 1,096.83 w m^{-2} in the summer, with average annual temperature of 22°C.

Forty-seven nulliparous female sheep were naturally infected with gastrointestinal parasites. They were between eight and twelve months of age and had an average weight of 44; 49 and 66 kg for the following breeds Santa Ines (n = 16), Texel (n = 16) and Ile de France (n = 15), respectively.

Animals were randomly assigned to each group, in both treatments, differentiated by the levels of crude protein (CP) shown in Table 1. The animals in the Treatment 1 (T1) received diet recommended by the NRC (2007) containing 60% total digestible nutrients (TDN) and 12% CP; animals of the Treatment 2 (T2) received a diet containing 60% TDN and 16% CP. Dry matter intake was estimated at 2.5% body weight of the animal.

Table 1. Chemical Composition of the ingredients and proximate composition of the diet given to ovine females during the experiment.

Ingredients	Chemical Composition		Proximate Composition of the diet (DM)	
	DM^1 (%)	CP^2 (%DM)	T1 (12% CP)	T2(16% CP)
Aruana Grass	53.32	2.56	67.21	71.68
Soybean meal	89.00	50.00	18.61	28.32
Cassava Starch residue	12.00	3.00	14.18	--

^{1} DM = Dry Matter; ^{2} CP = Crude Protein.

Animals were kept on three pastures of 1 hectare each with Aruana grass (*Panicum maximum* cv. IZ-5) in a rotation system during the day. The larvae contamination analysis indicated 75% of *Haemonchus* sp. and 25% of *Triclostrongilus* sp. At night they were taken to appropriate accomodation with a raised slatted floor.

From 7 hours 30 min. to 9 hours, the animals of the group that received diet with 12% crude

protein were fed with soybean meal on a forage: concentrate ratio of 81.39:18.61, while the animals of the group that received 16% crude protein were also fed with soybean meal but on a forage: concentrate ratio of 71.68:28.32. In the evening, the animals that received 12% CP were taken to the pen and received cassava starch at a proportion of 14.18% dry matter of the total diet in order to meet the requirements of NDT and maintain the 12% CP of the T1. Mineral salt was provided *ad libitum* with assurance levels per kg: 220 g Calcium, 130 g Phosphorus, 25.5 g Magnesium, 24 g Sulfur, 3,000 mg Iron, 1,500 mg Manganese, 4,000 mg Zinc, 1,200 mg Copper, 280 mg Cobalt, 260 mg Iodine, 30 mg Selenium and 300 mg Fluoride.

The animals remained on the same pasture throughout the month. The pasture rotation occurred on the first day of each month. The pasture used was fenced for two months after the females have left the area.

Sheep were wormed and vaccinated against clostridiosis thirty days before starting data collection, and monthly weighed to adjust the amount of amount of food for meeting nutritional levels recommended.

Fortnightly, the hematocrit level was determined and the FAMACHA© method was applied to each animal to classify the animals as proposed, in order to relate the FAMACHA© with the hematocrit value in ovine females of different breeds supplied with two different levels of crude protein.

Data were collected by comparing the color of the ocular mucous of each animal with the FAMACHA© card (VAN WYK; BATH, 2002). Seventeen data collections were performed during nine months of observations. Only one assessor, well-trained and capable of using the method, carried out all the assessments. Table 2 lists the hematocrit levels, according to the FAMACHA© values and the recommendation concerning to the need to worm the animals.

Table 2. Levels of hematocrit and worming recommendation according to FAMACHA©.

Classification by FAMACHA©	Hematocrit (%)	Color of the ocular mucous	Worming
1	≥ 28	Bright Red	No
2	$23 \leq x \leq 27$	Pale pink - Red	No
3	$18 \leq x \leq 22$	Pale Pink	Yes
4	$13 \leq x \leq 17$	Pale Pink - White	Yes
5	≤ 12	White	Yes

After all the assessments, blood samples from all the animals were taken by puncturing the jugular vein using a 18G x 1 1/2" hypodermic needle. The blood was collected in sterilized test-tubes with the anticoagulant *Ethylenediaminetetraacetic* (EDTA) and taken to a laboratory to determine the level of hematocrit.

Blood samples were placed in a microhematocrit centrifuge (Micro Haematocrit, Model KHT-400) for five min. at 14.490 X g. After the centrifugation, the capillaries were compared with a standard table obtain the hematocrit level.

During the experiment, all the animals with hematocrit levels below 18% were treated with moxidectin as active ingredient at 0.2 mg kg^{-1} administered by s.c. route in the thigh. The hematocrit level of 18% was set by Vatta et al. (2001) in order to conduct similar experiments and to provide a certain safety to the life of the animal.

The correlation between the hematocrit value with FAMACHA© method was determined to identify the accuracy of this method (Table 3). Binomial Analysis was employed to evaluate the degree of accuracy relative to correct or incorrect treatments, based on FAMACHA© anemia guide.

Table 3. Data classification according to accuracy of FAMACHA©.

Hematocrit (%)	Classification according to FAMACHA©	
	1 to 2	3 to 4 to 5
> 22%	right	wrong
< 22%	wrong	right

The method of generalized linear models was used for this analysis, with the aid of the software GLIM 4.0. It was assumed a binomial probability distribution function and logarithmic link function. The hypotheses were tested by Fisher's test and the means were compared using Tukey's post hoc test at 5% probability.

Results and discussion

The classification of the animals as to the hematophagous parasites by the FAMACHA© method, in percentage, was calculated by summing all the observations of the month (Figure 1). Based on the results, animals were classified into the level 3 only in September 2005. Although the method recommends treating the animals classified from level 3 of ocular mucous color, it was not carried out, due to the previously established criterion that only animals with a hematocrit value equal to or lower than 18% would be treated.
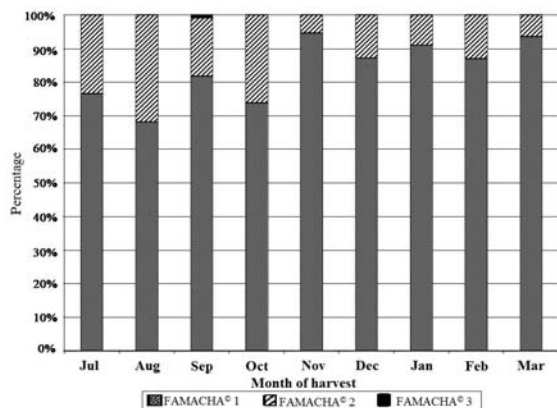
Figure 1. Identification of parasitological state of female sheep of different breeds with different weight in different periods by means of the FAMACHA© method.

When the classification by the FAMACHA© method is adjusted according to the hematocrit value (Figure 2) obtained from laboratory analysis, there is a slight change in the results.
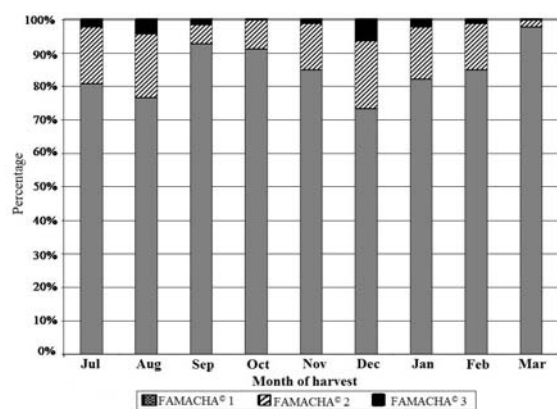
Figure 2. Prediction of the classification of female sheep by the FAMACHA© method according to the hematocrit value obtained during the experiment in 2005/2006.

A significant difference ($p < 0.05$) between breeds was observed when analyzed the number of accuracy and error between the observed and expected observations (Table 4).

Table 4. Percentage of errors found on the degree of anemia by the FAMACHA© method for the breeds Santa Ines, Texel and Ile de France.

Breed	Incidence of error
Santa Ines	10.50[a]
Texel	23.08[b]
Ile de France	8.92[a]

Values followed by different letters are significantly different by Turkey's test ($p < 0.05$).

The incidence of errors when evaluating the animals of the breed Texel was higher compared to the animals of Santa Ines and Ile de France breeds. Biologically, it was expected a higher incidence of errors to the breed Ile de France, which is more

susceptible to infection with *Haemonchus*. The variation in hematocrit values and the classification according to the FAMACHA© method was lower for Santa Ines and Ile de France breeds during the experiment. The greatest fluctuation of hematocrit levels was verified for Texel female lambs and resulted in a greater percentage of error when these sheep were evaluated. Kaplan et al. (2004) compared the percentage of correct and incorrect treatments by using the FAMACHA© classification and observed an error of 38%. Van Wyk and Bath (2002) reported an accuracy rate between 43% and 59% in the prediction of the hematocrit range and even though the rate had been expressive, it is because the animals are found in the transition zone, between one classification and another adjacent.

When working with mixed breed sheep and naturally infected on farmers located in the Campania region of southern Italy to measure the applicability of the FAMACHA© method as a strategy in the control of gastrointestinal helminth, Loria et al. (2009) observed that 92% and 87% of animals were correctly classified to be treated or not according to the FAMACHA© levels 4 and 5 or 3.4 and 5 respectively compared with hameatocrit, classified in sensitivity, specificity and negative predictive value (NPV). Positive predictive value (PPV) was always below 54%. The authors noticed that FAMACHA© represent an additional element that can integrate clinical examinations.

The results obtained in this study on the treatments and interaction between breeds and treatments are listed in Table 5.

Table 5. Percentage of error found in the assessment of anemia level by the FAMACHA© method in female sheep fed with different levels of crude protein and interactions between treatments and breeds.

% CP[1]	% of errors	% of errors in interactions between treatments and breeds		
		Santa Ines	Texel	Ile de France
T1 - 12	13.92[a]	10.92[a]	25.44[a]	5.88[a]
T2 - 16	14.53[a]	10.08[a]	20.83[a]	12.38[a]

[1]CP = Crude Protein; No significant differences ($p > 0.05$) were detected by Tukey's test.

There were no significant differences between treatments or interaction between breeds and protein levels. These results indicate that the crude protein content of 16% was not able to modify the FAMACHA© classification when compared to a content of 12% crude protein. Bricarello et al. (2005) reported that animals supplemented with protein showed a lower incidence of anemia. However, these authors worked with crude protein content of 10.2% and 17.2%.

The small variation in the FAMACHA© values can be related to the management of the animals. Female

lambs remained in the pen until 9 am when they were taken to the pasture. Yamamoto et al. (2004) studied the larval movement in the pasture and reported the occurrence of negative phototropism. These authors also observed that the amount of infective larvae in the upper third of the pasture was significantly smaller four hours after sunrise, indicating the importance of a pasture with high dry matter availability, which can reduce contaminant larvae in a selective grazing.

The correlation between the FAMACHA© method and the hematocrit value was 1:0.7991. This value is similar to that obtained by Molento et al. (2004), indicating that FAMACHA© has a good correlation with the hematocrit and can be safely used on the identification of animals with clinical signs of anemia.

The FAMACHA© method points out only infections caused by hematophagous parasites and the main helminth identified in sheep husbandry is *Haemonchus contortus*. A parallel monitoring to quantify these parasites should be used in combination with the FAMACHA© method to prevent non-hematophagous parasites causing damage to sheep production.

Conclusion

The FAMACHA© method can be recommended as a tool for monitoring the Haemonchosis of young female sheep, Santa Ines, Texel and Ile de France, in the northwestern Paraná State for being efficient to indicate the degree of anemia, thus reducing the frequency of deworming animals.

Since there was no difference in the FAMACHA© method influenced by the levels of 12% and 16% crude protein in the diet supplied to female sheep Santa Ines, Texel and Ile de France in the growth phase, it is recommended the lower level diet, with reduced commercial cost.

References

ABRÃO, D. C.; ABRÃO, S.; VIANA, C. H. C.; VALLE, C. R. Utilização do método Famacha no diagnóstico clínico individual de haemoncose em ovinos no Sudoeste do Estado de Minas Gerais. **Revista Brasileira de Parasitologia Veterinária**, v. 19, n. 1, p. 68-70, 2010.

BRICARELLO, P. A.; AMARANTE, A. F. T.; ROCHA, R. A.; CABRAL FILHO, S. L.; HUNTLEY, J. F.; HOUDIJK, J. G. M.; ABDALLA, A. L.; GENNARI, S. M. Influence of dietary protein supply on resistance to experimental infections with Haemonchus contortus in Ile de France and Santa Ines lambs. **Veterinary Parasitology**, v. 134, n. 1, p. 99-109, 2005.

BUZZULINI, C.; SILVA SOBRINHO, A. G.; COSTA, A. J.; SANTOS, T. R.; BORGES, F. A.; SOARES, V. E. Eficácia anti-helmíntica comparativa da associação albendazole, levamisole e ivermectina à moxidectina em ovinos. **Pesquisa Agropecuária Brasileira**, v. 42, n. 6, p. 891-895, 2007.

HAILE, A.; TEMBELY, S.; ANINDO, D. O.; MUKASA-MUGERWA, E.; REGE, J. E. O.; YAMI, A.; BAKER, R. L. Effects of breed and dietary protein supplementation on the responses to gastrointestinal nematode infections in Ethiopian sheep. **Small Ruminant Research**, v. 44, n. 3, p. 247-261, 2002.

KAPLAN, R. M.; BURKE, J. M.; TERRIL, T. H. Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. **Veterinary Parasitology**, v. 123, n. 1-2, p. 105-120, 2004.

LORIA, A.D.; VENEZIANO, V.; PIANTEDOSI, D.; RINALDI L.; CORTESE, L.; MEZZINO, L.; CRINGOLI, G.; CIAMARELLA, P. Evaluation of the FAMACHA© system for detecting the severity of anemia in sheep from southern Italy. **Veterinary Parasitology**, v. 161, n.1, p. 53-59, 2009.

MOLENTO, M. B.; TASCA, C.; GALLO, A. K.; FERREIRA, M. J.; BONONI, R. R.; STECCA, E. Método Famacha como parâmetro clínico individual de infecção por *Haemonchus contortus* em pequenos ruminantes. **Ciência Rural**, v. 34, n. 4, p. 1139-1145, 2004.

NRC-National Research Council. **Nutrient requirements of small ruminants**: Sheep, goats, cervids, and new world camelids. Washington, D.C.: National Academy Press, 2007.

RILEY, D. G.; VAN WYK, J. A. Genetic parameters for FAMACHA© score and related traits for host resistance/resilience and production at differing severities of worm challenge in a Merino flock in South Africa. **Veterinary Parasitology**, v. 164, n. 1, p. 44-52, 2009.

SOUZA, P.; BELLATO, V.; SARTORI, A. A.; RAMOS, C. I. Período para desinfestação das pastagens por larvas de nematoides gastrintestinais de ovinos, em condições naturais nos campos de Lages – SC. **Revista Brasileira Parasitologia Veterinária**, v. 9, n. 2, p. 159-164, 2000.

VAN HOULERT, M. F. J.; SYKES, A. R. Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. **International Journal for Parasitology**, v. 26, n. 11, p. 1151-1168, 1996.

VAN WYK, J. A.; BATH, G. F. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. **Veterinary Research**, v. 33, n. 5, p. 509-529, 2002.

VATTA, A. F.; LETTY, B. A.; VAN DER LINDE, M. J.; VAN WIJK, E. F.; HANSEN, J. W.; KRECEK, R. C. Testing for clinical anaemia caused by *Haemonchus* spp. In goats farmed under resource-poor conditions in South Africa using an eye colour chart developed for sheep. **Veterinary Parasitology**, v. 99, n. 1, p. 1-14, 2001.

VELOSO, C. F. M.; LOUVANDINI, H.; KIMURA, E. A.; AZEVEDO, C. R.; ENOKI, D. R.; FRANÇA, L. D.; MCMANUS, C. M.; DELL'PORTO, A.; SANTANA, A. P. Efeitos da suplementação protéica no controle da verminose e nas características de carcaça de ovinos Santa

Inês. **Ciência Animal Brasileira**, v. 5, n. 3, p. 131-139, 2006.

YAMAMOTO, S. M.; MACEDO, F. A. F.; GRANDE, P. A.; MARTINS, E. N.; ZUNDT, M.; MEXIA, A. A.; MARTIN NIETO, L. Produção e contaminação por helmintos parasitos de ovinos, em forrageiras de diferentes hábitos de crescimento. **Acta Scientiarum. Animal Sciences**, v. 26, n. 3, p. 379-384, 2004.