Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 12(3): 1274-1285, June 2018

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Resistance of Djallonké sheep to *Haemonchus contortus* under artificial challenge

Albert SOUDRÉ[1*], Moustapha GRÉMA[2], R. David NOTTER[3], A. R. Stéphane TAPSOBA[4], Amadou TRAORÉ[4], Adama KABORÉ[4], Isabel ALVAREZ[5], Ivan FERNANDEZ[5], Moumouni SANOU[4], Danani LOMPO[6], Teredié SANOU[6], Mohammed SAMSHUDDIN[7], Kathiravan PERIASAMY[7], Hamidou Hamadou TAMBOURA[4] and Félix GOYACHE[5]

[1] *Université Norbert Zongo, Unité de Formation et de Recherches en Sciences et Technologie (UFR/ST), BP 376 Koudougou, Burkina Faso.*
[2] *Faculté des Sciences Agronomiques Université de Diffa, BP 78, Diffa, Niger.*
[3] *Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA 24061, USA.*
[4] *Institut de l'Environnement et de Recherches Agricoles (INERA)-Laboratoire de Biologie et Santé animales, 04 BP 8645 Ouagadougou 04 Burkina Faso.*
[5] *Area de Genetica y Reproduccion Animal, Centro de Biotechnologia Animal, Serida Deva, Camino de Rioseco 1225, E33394 Gijón (Asturias), Spain.*
[6] *Institut Polytechnique Rural/Institut de Formation et de Recherches Appliquées de Katibougou, Bamako, Mali, IPR-IFRA, BP 06 Koulikoro, Bamako, Mali.*
[7] *International Atomic Energy Agency, Vienna International Centre, PO Box 100, 1400 Vienna, Austria.*
[*] *Corresponding author; E-mail: asoudre@gmail.com; Tel: (+226) 70256935*

ABSTRACT

Gastrointestinal parasitism is a major constraint for small ruminant production. Coupled to the development of drug resistance in parasite populations, it is of high interest to identify animals with tolerance to nematode infections. In this study we evaluated Djallonké and Sahelian sheep resistance to *Haemonchus contortus* infection in Burkina Faso by challenging a total of 35 lambs (21 Djallonké and 14 Sahelian). Five weeks following deworming, a single dose of 5,000 L3 infective larvae of *H. contortus* were inoculated to each lamb. Body weight (BW), fecal egg counts (FEC), packed cell volume (PCV) and FAffa Malan CHArt (FAMACHA) eye scores were recorded at days 0; 28; 35 and 42 following artificial infection. The body weight between days 28 and 42, the FAMACHA scores, PCV, FEC and lnFEC28-lnFEC35 were significantly correlated. Mean BW of Djallonké did not change significantly throughout the experiment while a significant loss of weight (p=0.04) was observed in Sahelian sheep. There were no breed effects on PCV, FEC and lnFEC (p>0.05), while effects of sex and measurement time on PCV and FAMACHA scores were significant (p<0.05), i.e., in Djallonké the FEC declined from 3945.14 to 3235.71 from days 28 to 35, and remained constant thereafter. The results of this study indicate that Djallonké breed is able to maintain its body weight despite the parasite burden. Together with the high variability found in FEC and PCV, Djallonké breed might be considered in selection program for internal parasite resistance.

Keywords: *Haemonchus contortus*, Sheep, FaffaMalanChart, Parasitism, Burkina Faso.

 6003-IJBCS
DOI: https://dx.doi.org/10.4314/ijbcs.v12i3.16

INTRODUCTION

The sheep population of Burkina Faso is estimated at 6.7 million with an annual growth rate of 3% (MRA, 2011). Sheep are widely distributed in the country providing a full range of useful products including meat, milk, skin and hair, which are of major economical importance for the maintenance of rural population and alleviate the effects of poverty all over the country.

In Burkina Faso, domestic sheep production system is mostly pastoral and traditional; thus animals are constantly exposed to gastrointestinal nematodes (GIN) infections leading to production losses and even death. In general, GIN infections and have been identified as one of the main problems affecting small ruminant productivity (Belem et al., 2005; Kaboré et al., 2007; Azando et al., 2011; Okombe et al., 2013; Minaflinou Sacca Sidi et al., 2015; Zabré et al., 2017). The control of nematodes traditionally relies on grazing management, anthelmintic treatment, or both. However, anthelminthic use is costly (MRA, 2012) and grazing management schemes are often impractical due to the hardiness and extent of infective larvae on the pasture, but also because of grazing ground loss to agricultural intensive systems.

The detection of genetically mediated resistance to anthelmintics in nematode populations (Jacquiet et al., 2009) has led to an increased interest in identifying animals with the ability to resist or tolerate nematodes infections. There is a well-documented evidence for within- and between-breeds genetic variation for resistance to GIN infections. The best documented research on sheep breeds showing resistance to endoparasites in Africa are the Red Maasai sheep in East Africa and the Djallonké sheep in West Africa (Baker et al., 2003). The major advantage of genetic selection to parasitic resistance is the production of animals that require fewer medicines, and thus lowering the cost of treatments while reducing anthelmintic contaminations in meat products and the environment.

Earlier studies identified markers for disease resistance in sheep with high variability among individuals in terms of disease susceptibility/tolerance. In natural population there has been found an association beween high genetic heterozygosity and reduced risk of infection (Luikart et al., 2008; Rijks et al., 2008). However, never there has been an infecion experiment been performed in a natural popualtion. Thus, Burkinabe sheep can be more or less considered as natural populaiton because they are not managed, not selected, often parasitized by several GIN species.

The purpose of this study was to evaluate the resistance of Djallonké and Sahelian sheep breeds taking into account phenotypes related to parasite infections of Burkina Faso to *Haemonchus contortus* after inoculation of a single dose of 5,000 infective L3 larvae under standardized experimental condition.

MATERIAL AND METHODS

Experimental site

The infection trial was conducted at the research station of *Direction Régionale des Recherches Environnementale et Agricole du Centre (DRREA-C), Saria*, Western centre, Burkina Faso. This site belongs to the Sudan-Sahel area, which is a transitional zone from Sahelian to Savannah with regards to rainfall and temperature, covering the central part of the country (roughly from latitude 11° 3′ N to 13° 5′ N), with a short rainy season from June to September and very variable rainfall with average of 750 mm per year. Temperatures vary between 20 °C and 42 °C, and the vegetation, benefitting from better hydric conditions than the Sahelian area, tend eventually toward a clear forest in the Southwestern extreme of the domain.

Breed and management system

This study was carried out in two sheep breeds: Djallonké and Sahelian. Initially, 25 Djallonké and 25 Sahelian lambs (two of each breed died before the trial), at about 5 months age, were bought from farmers in their original home area and were brought to the INERA research center located at Saria, Koudougou, Western centre Burkina Faso. Due to mortality during the trial, finally 21 Djallonké lambs and 14 Sahelian lambs remained at the end of the experiment. The Djallonké breed located in Sudan environmental area is short-eared and small-horned belonging to the West African dwarf sheep population. The Sahelian breed is a representative of the long-legged sheep group spread throughout the Sahelian region of West Africa, from Western Sudan in the east to Mauritania and Senegal in the west (Traoré et al., 2008).

After a one-week adaptation period, lambs were dewormed with levamisole (manufacturer's recommendation: 7.5 mg/kg) HCl to free them from internal parasites. To determine the effectiveness of deworming, FECs from 10% of lambs were performed at day 10 after deworming and found to be zero. The experimental animal were maintained during the whole trial in a dry lot and fed with dry forage and commercial concentrated feed to avoid re-infection with natural occurring GINs.

Infective *Haemonchus contortus* larvae production and inoculation

H. contortus adult worms were obtained from the abomasum of slaughtered sheep in Ouagadougou. The adult worms were collected and washed several times with PBS. Faeces from sheep were diluted in tap water and filtered. The decoction was poured into a petri dish in a layer of about 1-3 mm. The petri dish was incubated at 26 °C for 10 to 14 days. The recovered larvae were inoculated to two donor sheep, which were used as a source of *H. contortus* L3 covering the complete experiment with their faecal cultures, which were made following Hansen and Perry (1994). The infective third-stage larvae were aspirated by a pipette into marked vials and preserved at 4°C until inoculation. From the recovered L3 larvae, 5,000 were inoculated *per os* to individual lambs five weeks after deworming.

Data collection

Body weight (BW), faecal egg count (FEC), packed cell volume (PCV) and FAffa Malan CHArt (FAMACHA) eye scores were recorded at days 0 (day of infection with *H. contortus* larvae), 28, 35 and 42. Blood samples were collected with EDTA vacutainer tubes by jugular vein puncture at days of recording for PCV measurement. For body weight measurements, we used a 50 kg weighbridge with a sensitivity of 50 g. Faecal samples were obtained from the rectum and examined using the modified McMaster technique (Whitlock, 1948) that involves a saturated sodium chloride solution (sg1.20) with a sensitivity of 50 for an egg. PCV was measured after centrifugation of the whole blood at 8500g and the value was determined according to Murray et al. (1983). FAMACHA© scores were determined by using the recommended eye colour chart (Van wyk and Bath, 2002), ranged from 1 (normal, red colour of conjunctiva) to 5 (highly anaemic, pale coloration of conjunctiva).

Statistical analyses

lnFEC and BW were fitted by regression models for longitudinal data using the following repeated measures linear mixed model:

$$y \sim mean + time + breed + breed*time + animal + error$$

Where y represents the vector of repeated records for either lnFEC, PCV or BW. Time, breed and the interaction between breed and time were treated as fixed effects. The random animal effects were assumed independent and normally distributed. Maximum likelihood estimates of variance components and fixed and random effects were obtained using the HGLM package in R (R Core Team, 2015). Analysis of variance (ANOVA) tables and F-statistics were used to assess the significance of the fixed effects. The significance of the random effects was assessed using a likelihood ratio test comparing the full model against a null model without the animal effects. Finally, repeatability was measured as the variance explained by animal effects, *i.e.*, the ratio between the animal variance and the total variance.

Assuming FAMACHA scores as a categorization of a latent quantitative variable (i.e., the degree of paleness of the conjunctiva), we fitted a generalized linear mixed model with a cumulative probit link function between predictors and responses. The set of predictors used for the analysis was the same as for lnFEC, PCV and BW. Estimates of model parameters were obtained via maximum likelihood using the ordinal package in R (R Core Team, 2015). Differences were considered significant at $p<0.05$.

RESULTS

In this study we investigated the response of two West African sheep breeds to experimental GIN infection in order to identify breeds with a high natural (genetic) resistance.

Lamb mortality and changes in Body weight, packed cell volume and faecal egg count

Lamb mortality. The overall mortality rate during the trial was 8.69% in Djallonké and 39.13% in Sahelian breeds. Lambs that died were not necropsied. In the Sahelian breed, six lambs died from day 0 to up to day 28, two between days 28 and 35, and one between the final days 35 and 42. The dead Sahelian lambs were lighter (13.4±2.2 kg) than their surviving counterparts. The FEC values were lesser at day 28 (1100±141.4) than that counted at day 35 (3450±70.71). Better survival was found in Djallonké, only two lambs died during the whole trial with average BWs of 9.35, 8.4 and 7.6 kg at days 0, 28 and 35, respectively. Their PCV values ranged from 35 to 20 and the FEC values were 2300 and 3200, respectively, on days 28 and 35 after the challenge. These counts were lower than the values of the surviving lambs. The complete descriptive statistics of BW, PCV, FEC by breed, sex and time are summarised in Table 1.

Body weight. Overall, the effect of breed on BW was significant ($p<0.0001$), while the effects of sex and measurement times were not significant ($p>0.05$). Generally, Sahelian sheep were heavier than Djallonké sheep.

PCVs and FECs. The breeds did not affect PCVs and FECs, although the pattern showed the highest PCV (25.05± 9.7) and FEC values in Djallonké sheep (Table 1). Sex effect was significant on PCV and FEC ($p<0.0001$), i.e., PCV values were higher in female than in male lambs, while FECs were lower in females.

Throughout the trial, as a whole, BW remained constant from the day of challenge to day 35 and declined significantly at day 42. PCV and FEC declined markedly throughout the trial.

Breed and time interactions on BW, PCV and FEC

The breed and time interaction on BW is shown in Figure 1. Body weight for Djallonké (10.39, 10.16, 10.25 and 10.19 respectively at days 0, 28, 35 and 42) remained constant throughout the experiment but below those of Sahelian. This superiority of Sahelian was maintained during the whole trial but a loss of weight was observed in the breed at the end of the trial ($p<0.0001$).

Breed and time interactions on PCV, FEC and lnFEC are reported in Table 2. In both breeds, FEC at the day of challenge were 0 indicating the effectiveness of deworming. In Djallonké, the mean FEC values has increased quickly from day 0 to day 28 (0 to 3945.14), declined from day 28 to day 35 (3945.14 to 3235.71) and increased again at day 42 (3557.14) but less than the values observed at day 28. This pattern was different in Sahelian where FEC values increased continuously throughout the trial, from day 0 up to day 42. However, the observed values were lower in Sahelian.

On the day of challenge, the mean PCV values were 33.76±7.38 (21-48) in Djallonké and 26.64±4.12 (20-33) in Sahelian sheep. In both breeds, the means PCV values declined throughout the trial (18.19±6.86 and 20.71±8.90 respectively in Djallonké and Sahelian).

For both FEC and PCV, the standard deviations were very high indicating a substantial variability for these two phenotypic parameters. Considering FEC, this variation was higher in Djallonké compared with Sahelian at each measurement time. For PCV, the variation was higher at day 0 and day 28 in Djallonke.

FAMACHA scores

Frequencies of FAMACHA scores at each measurement time

The frequencies of FAMACHA scores, fitted by measurement times are presented in Figure 2. On the day of challenge, only FAMACHA scores 1 and 2 were observed with respective frequencies of 65.7% and 34.3%. At days 28 and 35, the most frequent FAMACHA scores were 2 and 3. The highest FAMACHA scores 4 and 5 were found at day 42 after challenging. Least square means of FAMACHA scores revealed a significant effect of measurement time on FAMACHA scores ($p<0.001$).

Frequencies of FAMACHA scores by breed within measurement times

The frequencies of FAMACHA scores fitted by breed at each measurement times are presented in Figure 3. At day 0 (Figure 3a) FAMACHA score in Djallonké was 1. It increased with the time course up to 4 (day 42; Figures 3b to 3d) in Djallonké sheep. The highest FAMACHA scores were found in the Sahelian breed (Figure 3d).

Faecal egg count and associated FAMACHA eye scores category

Feacal egg count values and their corresponding FAMACHA scores per breed are given in Table 3. Our results showed a progressive increase in mean FEC values with the increase of FAMACHA scores. The correlation between FAMACHA score and FEC was positive and highly significant ($p<0.0001$). FAMACHA score 1 corresponded to animal with the lowest FEC values, while those sheep with the highest FEC values had score 4 in Djallonké and 5 in Sahelian. However, for all the FAMACHA score considered, the standard deviation of FEC was high and different from the mean FEC values accepted for FAMACHA scores 3, 4 and 5 in both breeds.

The increase of FEC values coincides with the progressive pallor of the eye mucous membrane due to parasites in the bloodstream like *Haemonchus contortus.*

PCV values and associated FAMACHA scores

PCV values and their corresponding FAMACHA scores are given in Table 4. The correlation between FAMACHA scores and PCV values was negative (-0.47) and highly significant ($p<0.0001$). Animals with the highest PCV values had the FAMACHA score 1 and those with the lowest PCV values had score 4 in Djallonké and 5 in Sahelian.

Effects of FAMACHA scores on BW

BW values and their corresponding FAMACHA scores are presented in Figure 4. In both Djallonké and Sahelian, BW decreased with the increase of FAMACHA eye scores.

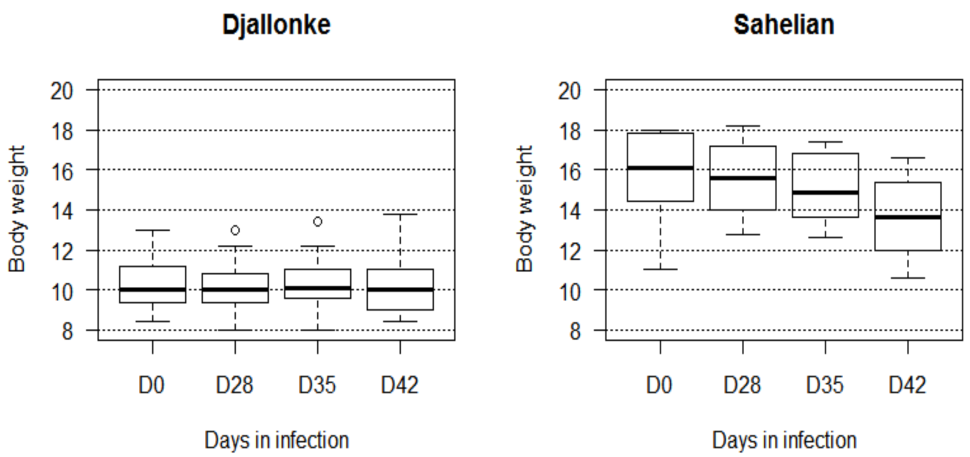

Figure 1: Time interaction on Body weight (BW) of Djallonké and Sahelian breeds.
D0: day 0.

Table 1: Means (standard deviations) of body weight, packed cell volume and faecal egg count computed by breed, sex and measurement time.

Variables	BW	PCV	FEC
Breed	S	NS	NS
Djallonké	10.3[a] (1.34)	25.05[a] (9.7)	2685[a] (3925)
Sahelian	15[b] (2.01)	23.01[a] (7.4)	1620[a] (1383)
Sex	NS	S	S
Male	12.5[a] (2.9)	22.73[a] (8.3)	2987.8[a] (3729.7)
Female	11.85[a] (2.8)	25.66[b] (9.2)	1569.8[b] (2430.7)
Time	S	S	S
D0	12.48[a] (3.1)	30.9[a] (7.1)	0.00
D28	12.34[a] (3.05)	24.3[b] (7.5)	4102.7[a] (1227.5)
D35	12.19[a] (2.9)	22.6[c] (8.4)	2947.05[b] (2090)
D42	11.57[b] (2.3)	19.2[d] (7.7)	3008.3[c] (2402.5)

BW: body weight; PCV: packed cell volume; FEC: faecal egg counts. D28: Day 28. Difference is statistically significant (S), not significant (NS). Different lowercase letters in same column mean statistically different.

Table 2: Effect of breed and time interactions on PCV, FEC and lnFEC.

Breed : time	PCV	FEC	lnFEC
	LSM	LSM	LSM
Djallonké	S	S	S
Djallonké_0	33.76[a] (7.38)	0.00	-
Djallonké_28	25.14[b] (9.27)	3945.14[a] (4962.35)	7.41[a] (1.43)
Djallonké_35	23.14[c] (8.27)	3235.71[b] (3745.91)	7.24 [b] (1.46)
Djallonké_42	18.19[d] (6.86)	3557.14[b] (3862.43)	7.48 [b] (1.35)
Sahelian			
Sahelian_0	26.64[a] (4.12)	0.00	-
Sahelian_28	23.00[a] (5.65)	914.28[a] (448.68)	6.70 (0.6)
Sahelian_35	21.71[a] (8.88)	2392.85[b] (817.56)	7.72 (0.38)
Sahelian_042	20.71[b] (8.90)	3171.42[c] (787.81)	8.03 (0.26)

BW: body weight; PCV: packed cell volume; FEC: faecal egg counts. LSM: least squares mean. Djallonké_28: Djallonké Day 28. Difference is statistically significant (S), not significant (NS). Different lowercase letters in same column mean statistically different.

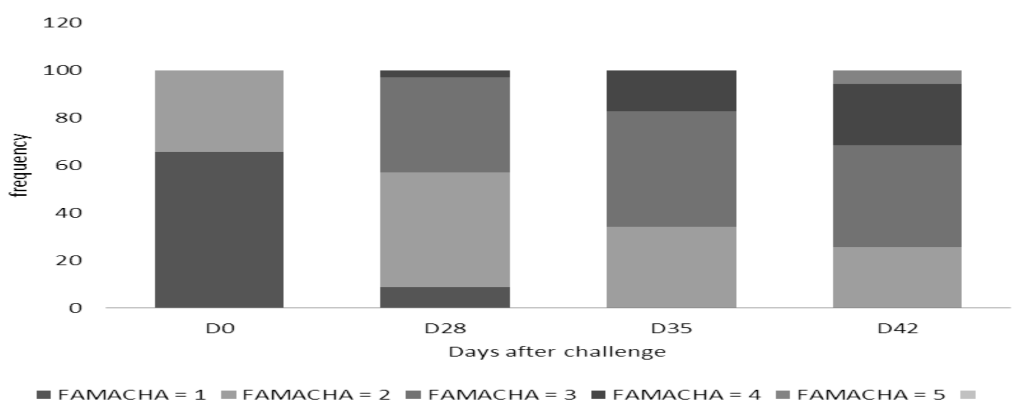

Figure 2: Frequencies of FAMACHA eye scores at each measurement time
D0: day 0

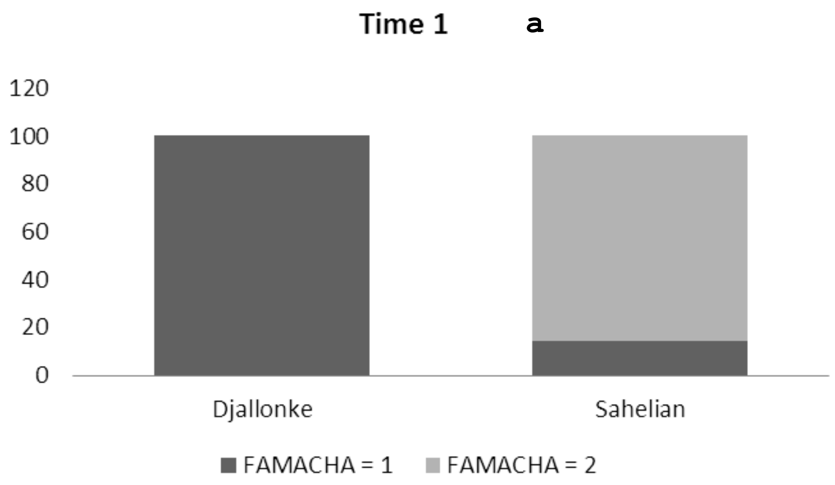

Figure 3a: Frequencies of FAMACHA scores by breed at day of challenge.

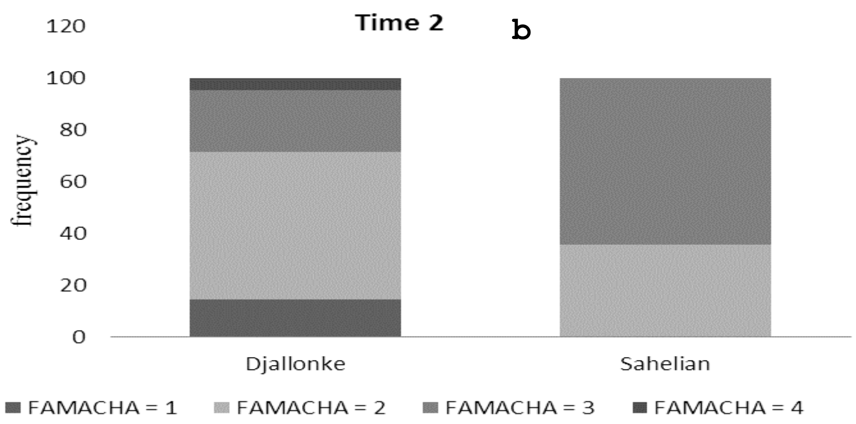

Figure 3b: Frequencies of FAMACHA scores by breed at day 28 after challenge.

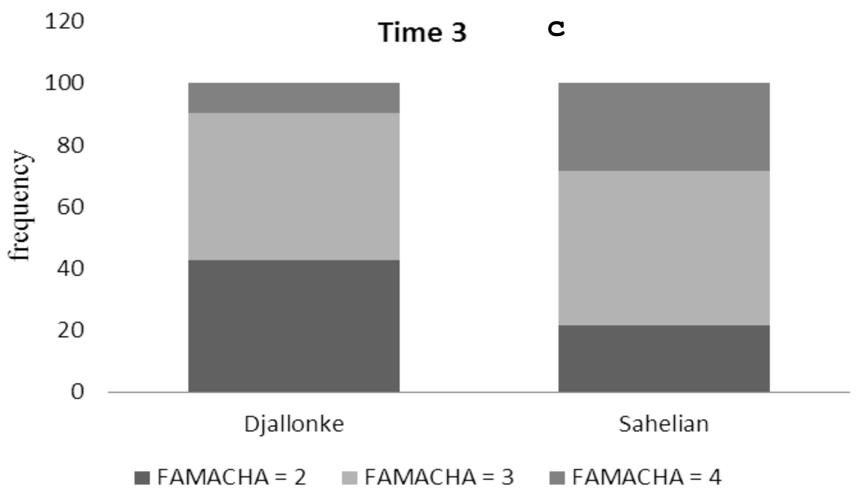

Figure 3c: Frequencies of FAMACHA scores by breed at day 35 after challenge.

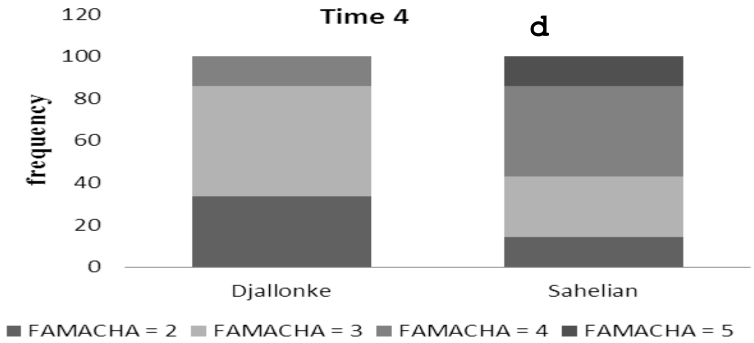

Figure 3d: Frequencies of FAMACHA scores by breed at day 42 after challenge.

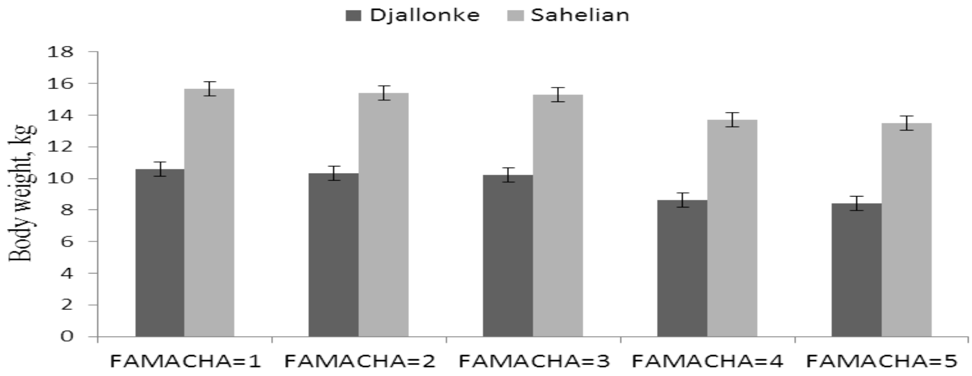

Figure 4: Effect of FAMACHA eye scores on BW by breed.

Table 3: Means and standard deviation of faecal egg count (eggs/g) by FAMACHA eye score category for Djallonké and Sahelian sheep.

FAMACHA scores	Djallonké		Sahelian	
	N	**Mean (s.d.)**	**N**	**Mean (s.d)**
1	24	341.25 (1517.16)	2	-
2	28	1873.50 (2901.86)	22	554.54 (727.50)
3	26	4648.84 (4568.93)	20	1895.00 (1039.97)
4	6	7330.00 (4390.91)	10	3280.00 (633.85)
5	-	-	2	3900.00 (424.26)

N: Sample size; (s.d.): standard deviation.

Table 4: Means and standard deviation of PCV values by FAMACHA eye score category for Djallonké and Sahelian sheep.

FAMACHA scores	Djallonké		Sahelian	
	N	**Mean (s.d.)**	**N**	**Mean (s.d.)**
1	24	31.95 (9.44)	2	33.00
2	28	24.14 (9.00)	22	25.00 (3.77)
3	26	21.42 (8.06)	20	23.25 (7.53)
4	6	17.50 (3.56)	10	18.80 (9.21)
5	-	-	2	10.00 (5.65)

N: Sample size; (s.d.): standard deviation.

DISCUSSION

Lamb mortality and changes in Body weight, packed cell volume and faecal egg count

Our results showed lamb mortality from the beginning to the end of the trial. Lamb mortality has been also reported by Traoré et al. (2017) in Comoé province of Burkina Faso, which was lower (5.5%) than the rate in our study for both breeds. The breed has had an effect on the BW. Indeed Sahelian sheep were heavier than Djallonké sheep. Our result is supported by previous studies on the two breeds in Burkina Faso (Traoré et al., 2008) and in Ghana (Birteeb et al., 2013) where all the morphological characters assessed were higher in Sahelian than in Djallonké sheep.

PCV values were higher in female than in male lambs, while FECs were lower in females, showing sex effect on PCV and FECs as previously reported by Razzaq et al. (2013). According to Urquhart et al. (1996), the sex effect on PCV and FEC might be related to androgen hormones in males, which could account for their susceptibility to parasite infection. Contrary, females are more resistant to infection due to the stimulatory effects of estrogen on immune response.

Body weight, PCV and FEC declined by the time course similarly to the finding of Getachew et al. (2015) in Ethiopia.

Breed and time interactions on BW, PCV and FEC

Our results suggested that Sahelian breed is affected by the parasite challenge while the experimental infection did not result in weight losses in Djallonké breed which were able to maintain its BW constant. Getachew et al. (2015) have also shown the same pattern in two Ethiopian breed where the Menz breed were able to maintain their body weight despite parasites challenge while Washera breed lost about 1 kg during the trial. Notter et al. (2003) reported similar pattern like that of Djallonké in hair and wool sheep after artificial challenge and have considered this results important if the animals are to be screened for parasite susceptibility.

Faecal egg count values were higher in Djallonké compared with Sahelian. Higher values could be associated with the consistent difference in BW between the two breeds, higher FEC generally associated with lighter BW as noted before by Vanimissetti et al. (2004).

The very high variability of the standard deviations for both FEC and PC reported in the current study indicates that no selection program was implemented in Burkina Faso sheep breeds giving a possibility for selection for parasites resistance particularly in Djallonké (Belem et al., 2005). Getachew et al. (2015) have reported the same finding in Ethiopian Menz and Washera sheep breeds.

FAMACHA scores

Frequencies of FAMACHA scores at each measurement time

Least square means of FAMACHA scores in our study revealed a significant effect of measurement time on FAMACHA scores ($p<0.001$). This result is consistent with that of Getachew et al. (2005). FAMACHA scores were positively correlated with FEC and negatively correlated with PCV during the whole trial. Similar findings were reported by Di Loria et al. (2009) in sheep from Southern Italy.

Frequencies of FAMACHA scores by breed within measurement times

Opposite to the results reported by Getachew et al. (2015), FAMACHA eye scores were affected by breed ($p<0.05$). Indeed the highest FAMACHA scores were found in the Sahelian sheep.

Faecal egg count and associated FAMACHA eye scores category

For all the FAMACHA score considered, the standard deviation of FEC is high and different from the mean FEC values accepted for FAMACHA scores 3, 4 and 5 in both breeds. Similar patterns were reported by several authors (Burke et al., 2007; Fondraz, 2012; Guash et al., 2015).

The increase of FAMACHA scores corresponds with progressive parasite infections. The highest FAMACHA scores

found in the Sahelian were due to the susceptibility of the breed to GIN parasites
It is a useful tool for small ruminant breeders to decide which individuals need anthelminthic treatment as previously suggested by Burke et al. (2007), Guash et al. (2015) and Getachew et al. (2015).

PCV values and associated FAMACHA scores

Assigned values of PCV for each FAMACHA scores were higher than the results reported by Papadopoulos et al. (2013) similar to the FEC values. This might mainly be due to the local epidemiological situation in Greece where the prevalence of *Haemonchus contortus* is low. The mean PCV values associated to individual FAMACHA Scores in the current work were consistent with those previously reported in West African sheep (Vatta et al., 2001; Van Wyk et Bath, 2002; Burke et al., 2007; Glaji et al., 2014).

Effects of FAMACHA scores on BW

The increase of FAMACHA scores corresponds to the increase of parasitic load, which has a negative influence on the BW (Nurzaty et al., 2014; Yimaz et al., 2014). Thus, BW can be an indicator of the general ability of the lamb to control their parasite burden.

Conclusion

The current research was undertaken to evaluate the resistance of Djallonké and Sahelian sheep breed to *Haemonchus contortus* using a single dose of 5,000 infective larvae. Throughout the trial, the BW of Sahelian remained superior to that of Djallonké. However, the artificial infection in this study did not result in BW losses in Djallonké breed, while the BW of Sahelian breed was affected by the increase of FEC value during the trial. Contrary to the BW, the FEC values were higher in Djallonké, where the mean FEC values increased quickly from day 0 to 28, declined from day 28 to day 35 and remained unchanged at the end of the trial; while the FECs of Sahelian continued to increase throughout the trial. PCV decreased in both breeds throughout the trial and the decline was similar in Djallonké and Sahelian. High variation in FECs and PCVs was observed in both breeds, but it was slightly higher in Djallonké. The correlations between repeated measures were positive and highly significant indicating a good quality of the experimental data. The increase of FAMACHA scores corresponded with the increase of FEC and the decline of PCV and BW values. The current results point out the best resistance of Djallonké breed to GIN infection and we suggest taking into account internal parasite in selection program for improvement, mainly in Djallonké sheep breed. Investigation is needed in future research to identify genetic markers associated with resistance to parasites diseases in Burkina Faso sheep breeds. The Djallonké sheep can further be used for selection and larger distribution in rural areas with limited access to veterinary treatment.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

AT, MS and KP designed the project; SART, AK, MS, DL, TS, and HHT collected breed information and samples; AS, KP, AT, IA, IF and FG performed analysis of data and wrote the manuscript.

ACKNOWLEDGEMENTS

This study was funded by Joint FAO/IAEA Coordinated Research Project D3.10.26 and by Fond Compétitif National of Burkina Faso (CNRST/PPAAO).

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