

## **RNRRS LIVESTOCK PRODUCTION PROGRAMME**

### **R6140 INFLUENCE OF NUTRITION ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF TRYPANOSOME-INFECTED SMALL RUMINANTS**

Project Leader: Dr I.A.Jeffcoate  
Department of Veterinary Physiology  
University of Glasgow Veterinary School

Overseas Collaborators Dr Sabine Osaer and Dr Bart Goossens  
ITC, The Gambia

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#### **Executive Summary**

The project purpose was to investigate the effects of trypanosome infection on aspects of reproductive performance in West African sheep and then to assess the value of nutritional supplementation in ameliorating observed constraints to productivity. Adult sheep were infected upon breeding and followed through conception until mid-lactation. Immature ewes were infected and followed through puberty until first lambing. Trypanosome infection had severely deleterious effects on pregnancy rates, with infected ewes showing many barren cycles before conception and pregnancy establishment. Moreover, this could not be ameliorated by better nutrition. Chronic effects of trypanosome infection later in pregnancy, at parturition and during lactation were much less serious and at these stages, nutritional supplements improved productivity in terms of lambing performance, lamb growth rates and lamb reproductive success. Trypanosome infection was not seen to have significant effects on age at puberty although age at first lambing was delayed. Nutritional supplements were beneficial on these traits but possibly through interaction with lamb liveweight. Again, nutritional supplementation had beneficial effects on lamb survival out of the infected animals.

In summary, nutritional supplements are recommended to improve productivity of trypanosome-infected ewes during pregnancy and lactation and of ewe lambs during the period of rapid growth before puberty. In case of likely exposure to trypanosome challenge during the breeding period and early pregnancy, ewes should be given prophylactic drugs as nutritional supplements are not protective. These measures should improve reproductive success and increase the lifetime reproductive potential of sheep in trypanosome-endemic regions, thereby increasing the value of farm stock.. These recommendations are in line with DFID's developmental goals of relieving suffering of the rural poor through poverty alleviation.

## **Background**

Sheep play a crucial role in West African farming systems both as a protein source and as an asset, particularly for women. They are also important for religious and ceremonial occasions. The demand for sheep is high and fortunately their reproductive potential is high, owing mainly to their precocity and the minimal influence of season and lactation on reproductive rate. As in all sub-Saharan African countries, however, sheep health and productivity is seriously compromised by disease, particularly trypanosomosis, and under-nutrition. Generally speaking, farmers and extensionists have paid little attention to these constraints in small ruminant production but both are now considered to be serious enough to have warranted some investigation. For example Agyemang (1991) and Katunguka (1993) have shown that nutritional status has marked effects on the pathogenic impact of trypanosomosis with decreases in both energy and protein intake being deleterious. Similarly much recent research is pointing to the diverse nature of trypanosome interference in the reproductive process (reviewed by Jeffcoate and Holmes 1997).

While native west African sheep are considered to possess considerable tolerance to trypanosomes and can survive and reproduce under high levels of parasitaemia and consequent anaemia (Osaer et al 1994), they require adequate nutrition for such resistance, yet interactions between nutritional requirements and reproduction have not been elucidated.

## **Project purpose**

To identify the chief constraints to maximising small ruminant reproductive potential caused by trypanosomosis and to determine the benefits of nutritional supplementation in alleviating these constraints.

The experiments were designed to investigate separate and combined effects of trypanosomosis and nutritional supplements on:

- a) Conception rate and maintenance of pregnancy
- b) Lambing rate and offspring survival and growth rate
- c) Age of ewe lambs at puberty and first lambing

## **Research Activities**

The research programme was mostly conducted at The International Trypanotolerance Centre (ITC), The Gambia; with the exception of some assay validation work which was carried out at Glasgow Veterinary School (GUVS) and some specialist assay services contracted out to Dr Humblot at UNCEIA Services Techniques, Maisons-Alfort, France. The project research activities fell under three main headings:

Experiment 1). *Impact of nutritional supplementation on reproductive function in trypanosome-infected West African ewes.*

Forty Djallonke ewes were allocated onto either high or low planes of nutrition (120g CP and 9 MJ/d vs 90g CP and 7 MJ/d respectively), starting about 7 weeks before oestrus synchronization using a standard progestagen pessary and PMSG procedure. Half the animals in each dietary block were infected with *Trypanosoma congolense*, from a well-described laboratory stock, in time for peak parasitaemia to occur at the time of expected oestrus. Four experimental groups of

ten ewes were thus created: High plane of nutrition Infected (HI); High plane uninfected Controls (HC); Low plane of nutrition Infected (LI) and Low plane uninfected Controls (LC). Blood samples were collected once weekly to monitor haematology parameters. There were three phases of activity in this experiment:

- 1) Oestrus detection and monitoring differences in circulating progesterone and the early pregnancy protein PSPB during the synchronised cycle. For this, additional blood was collected on days 7, 12, 17, 19, 21 and 26 for analysis of plasma progesterone by EIA and and early pregnancy protein (PSPB) by RIA.
- 2) Observations over the following 5 months to monitor repeat breeding and to check plasma progesterone profiles and ewe liveweight during pregnancy. For this blood sampling and liveweight measurements continued at weekly intervals throughout pregnancy.
- 3) Observation of ewe liveweight at parturition and lambing outcome and recording of lamb growth rates.  
For this, ewe liveweight was recorded until after parturition and lamb liveweight gain was calculated from weekly recordings of lamb weight

Experiment 2). *Impact of nutritional supplementation for West African ewe lambs on the onset of puberty.*

Twenty four ewe lambs at 4 months of age and weighing around 10 kg were allocated equally into dietary and Trypanosoma congolense-infected groups as above (i.e. HI, HC, LI and LC). The composition of the ration was identical to experiment 1 but High and Low groups were initially offered 115g CP and 6 MJ/d vs 66g CP and 3.7 MJ/d respectively, this being increased during the experiment in proportion to liveweight. Individual food intake was recorded daily and liveweight weekly. Blood samples collected weekly were analysed for parasitaemia and haematocrit, and plasma progesterone concentration, to detect increases associated with first ovulation, the working definition of puberty attainment. Colour-marked rams ran with the ewe lambs at all times to confirm oestrus times and for breeding purposes. These ewe lambs were observed closely until they were confirmed pregnant and from then until parturition.

Experiment 3). *Development and validation of a progesterone immunoassay.*

A double-antibody enzymimmunoassay (EIA) had been previously developed by H.Meyer in association with ITC for use in cattle. The procedure was modified somewhat and adapted and validated for use in sheep by the use of standards made up in castrate ram plasma.

The procedure was briefly as follows:

EIA plates were coated with anti-rat serum Sigma (R5130), 1 $\mu$ g/well. Standards (range 10 – 0.15ng/mL), control plasma or samples (20 $\mu$ L volume) were added to respective wells. For the assay, a rat progesterone antibody (Sigma Clone 2H4) was used (100 $\mu$ L per well, 1:100 dilution) together with a progesterone-peroxidase label (Sigma P3659, (100 $\mu$ L per well, 1:100 dilution)). Plates were covered and incubated for 2h. After washing, TMB substrate (150 $\mu$ L) was added to each well and colour allowed to develop for 20 minutes. The OD readings were taken and fed into a data analysis programme which was operated by ITC personnel. In our hands, the assay achieved consistently good standards of quality control with between and within assay coefficients of variation of about 15 and 7% respectively. The

progesterone standards were prepared at ITC and validated against both in-house and commercial preparations at GUVS and commercial EIA kits were occasionally used at ITC as another means of external quality control.

## Outputs

### Experiment 1.

Looking at the effects of trypanosome infection and nutritional supplementation on breeding, conception and early pregnancy; there were clear indications that severity of parasitaemia was not alleviated by supplementation ( $P < 0.05$ ).

Also, while trypanosome infection reduced conception rate and extended the time taken for ewes to get pregnant (i.e., there were increased numbers of barren cycles in the infected ewes), nutritional supplementation could not offer any benefit as shown below:

Group Treatment/diet	Interval (d) from induced oestrus to proven conception
LC	29.8 $\pm$ 17.2
LI	58.2 $\pm$ 19.1
HC	0.6 $\pm$ 11.5
HI	98.6 $\pm$ 48.9

With reference to the inability of infected ewes to conceive, our data shows a significant effect of trypanosome infection on plasma progesterone concentration during the first synchronised cycle when parasitaemia was developing (plasma progesterone was 4.3  $\pm$  17 ng/ml higher in control vs infected ewes at this time,  $P < 0.01$ ). There was also a tendency for plasma concentration of the early pregnancy protein PSPB to be lower in infected ewes (1.5  $\pm$  0.4 vs 2.3  $\pm$  0.2 ng/ml). While PSPB is only an indicator of successful pregnancy, an adequate level of progesterone is an absolute requirement for successful conception and implantation and these data show how serious trypanosome infections may be during the period of pregnancy establishment.

However, nutritional supplementation did have beneficial effects on both maintenance of and successful outcome of established pregnancy in trypanosome infected ewes. This was apparent despite the fact that ewe liveweight gains during pregnancy, which were attributable to nutritional supplementation, were significantly depressed by trypanosome infection ( $P < 0.01$ ). Diet also interacted significantly with infection and the offspring from the low plane infected (LI) ewes had lower growth rates as shown below:

Group treatment/diet	Offspring daily liveweight gain (g)
LC	45.2 $\pm$ 10.7 <sup>ac</sup>
LI	20.9 $\pm$ 8.1 <sup>c</sup>
HC	54.3 $\pm$ 5.9 <sup>a</sup>
HI	121.0 $\pm$ 19.7 <sup>b</sup>
Significance	$P < 0.01$

It is concluded that dietary supplementation of trypanosome-infected pregnant ewes can improve productivity in terms of protecting the ewe until parturition, and

ensuring better lamb growth rates to weaning. The latter effect suggests beneficial effects of nutritional supplements on milk yield and/or immunity acquired by the lamb from the milk.

#### Experiment 2.

Looking at the benefits of nutritional supplementation on puberty and age at first lambing in trypanosome-infected ewe lambs, simple indices of reproductive performance, we showed that trypanosome infection tended to delay age at first lambing and better nutrition significantly advanced puberty ( $P < 0.05$ ) as shown below:

Group treatment/diet	Age at puberty (d)	Age at lambing (d)
HC	236.5 $\pm$ 47.2	549.8 $\pm$ 20.5
HI	273.2 $\pm$ 47.2	579.6 $\pm$ 23.4
LC	353.3 $\pm$ 47.2	599.7 $\pm$ 20.5
LI	343.2 $\pm$ 47.2	632.9 $\pm$ 23.4
Overall supplemented	254.8 $\pm$ 33.4	565.7 $\pm$ 15.5
Overall non-supplemented	348.3 $\pm$ 33.4	616.3 $\pm$ 15.5
Overall non-infected	295	575
Overall infected	308	606

The interaction diet x infection was not significant with the effects only being additive (eg age at lambing: LI > LC > HI > HC). We have concluded that the effects of trypanosome infection on puberty were related more to depression of growth rate and that supplementary feeding had beneficial effects on reproductive performance in growing lambs.

In follow up studies with the offspring from these lambs we could show that lambs out of the low plane infected (LI) group had higher mortality rates up to 3 months of age ( $P < 0.01$ ) and that nutritional supplementation gave better survival rates.

#### Experiment 3.

We have demonstrated the usefulness of the progesterone EIA in assessing reproductive function in ewes and ewe lambs. The assay is reliable and robust enough to be used in the tropics and much more economical than commercial kits.

## Contribution of outputs

The goal of the project was met

- a) by providing original data on the influence of nutrition on the growth and reproductive performance of trypanosome-infected small ruminants
- and b) by development of strategies which will be useful to improve productivity of small ruminants in trypanosome-endemic areas.

Overall, the results of the project have shown that nutritional supplements are beneficial to the survival and productivity of trypanotolerant sheep such as the West African Djallonke. We have demonstrated that supplements improve several aspects of productivity such as:

- i) pregnancy outcome and lamb survival
- ii) lamb growth rates
- iii) rate of attainment of puberty

But nutritional supplementation is less important during the breeding period than trypanosome control measures

We have shown that nutritional supplements have a protective effect on established pregnancy and when they are given to lambs, both growth rate and survival to puberty are enhanced. Certain aspects of the reproductive process cannot be safeguarded from the detrimental effects of trypanosome infection, however, and these include conception rates and/or maintenance of early pregnancy. Taken together, the results of the project, the major outputs, show that the lifetime reproductive potential of Djallonke sheep, at risk from trypanosome infection, can be enhanced by nutritional supplementation. This should be reason enough to recommend giving supplements to pregnant and lactating ewes and also to growing lambs. Of added interest was the within group variation in response to trypanosome infection. Clearly some animals possess a greater degree of trypanotolerance than others. This suggests that a selective breeding programme might be of use in developing lines of Djallonke sheep with greater than normal trypanotolerance.

The outputs of the project are quite clear. It can be stated with certainty that nutritional supplements should be made available to sheep in trypanosome-endemic regions, particularly during pregnancy and lactation and also during the prepubertal period. Adherence to this guideline will maximise the chances of reproductive success and improve the lifetime reproductive performance of each ewe thus contributing to DFID's aims of poverty alleviation amongst the rural poor through sustainable improvements in animal production.

Promotion of the findings of this work has already been implemented through three different channels:

- 1) in the form of informal discussions with government agricultural extension officers in The Gambia
- 2) in the form of spoken and poster presentations at international and regional livestock science meetings
- 3) in the form of publications in international journals:

Several spin-offs from the main project have also been publishable, for example articles concerning oestrus synchronisation techniques, description of

blood biochemical changes in trypanosome-infected animals and comparative studies of Djallonke sheep, Sahel-Djallonke crossbreeds and West African goats.

### International Journals

**Osaer S, Goossens B, Jeffcoate IA, Jaitner J, Kora S and Holmes PH (1998)** Effects of *Trypanosoma congolense* and nutritional supplements on establishment and outcome of pregnancy in trypanotolerant Djallonke ewes. *Animal Reproduction Science* 51, 97-109.

**Osaer S, Goossens B, Jeffcoate IA and Holmes PH (1998)** Effects of *Trypanosoma congolense* and nutritional supplements in Djallonke ewes on liveweight during pregnancy, post partum weight, haematology parameters and lamb performance. *Research in Veterinary Science* 65, 65-69

**Osaer S, Goossens B, Kora S and Jeffcoate IA (1999)** Effects of *Trypanosoma congolense* infection and diet on puberty, age at first lambing and haematology changes in Djallonke ewe lambs. *Veterinary Parasitology* 80, 215-230

**Osaer S., Akinbamijo, A.A. and Goossens B.** Some biochemical changes following *Trypanosoma congolense* infection in Djallonké ewe lambs and breeding ewes fed on two levels of nutrition. Submitted to *Acta Tropica*.

### Conference proceedings

**Jeffcoate IA, Osaer S, Goossens B, Humblot P & Holmes PH (1996)** Effects of *Trypanosoma congolense* infection and nutritional supplements in sheep on early pregnancy rate assessed by measurement of progesterone and PSPB. *Journal of Reproduction and Fertility*, Abstr series 17, 56.

**Goossens, B. and Osaer, S. (1997)** Synchronisation techniques in Djallonke sheep : comparing the ram effect with drug mediated techniques. Presented at the 24 th meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) in Maputo - Mozambique 29 Sept - 03 Oct 1997.

**B. Goossens, Osaer S, Van Wingham J, Ndao M and Geerts S (1998)** Studies on health and productivity of trypanotolerant small ruminants under different trypanosomiasis risk. Presented at the 9th AITVM conference in Harare, Zimbabwe.

**Osaer S and Goossens B (1996)** Productivity and clinical effects in trypanotolerant sheep and goats following an artificial *Trypanosoma congolense* infection. Presented at the 4th biennial conference of the Small Ruminants Network for Africa (SRNET). ILRI, Addis Ababa, Ethiopia, 9-14 Dec 1996

**Osaer S, Goossens B, Van Wingham J, Ndoa M and Geerts S (1998)** The susceptibility of Djallonke sheep and their crosses with trypanosusceptible Sahelian breeds to experimental *Trypanosoma congolense* infection and interactions with nutritional status and natural helminth infection. Presented at the 9th AITVM conference in Harare, Zimbabwe.

**Osaer S, Goossens B and Jeffcoate I (1998)** Effects of diet and *Trypanosoma congolense* infection on puberty, age at first lambing and the establishment and outcome of pregnancy in Djallonké ewes. Presented at the 9th symposium on Tropical Animal Health and Production. University of Utrecht, Faculty of Vet. Medicine, 27 Nov.1998.