THE EFFECT OF PLANE OF NUTRITION ON PATHOPHYSIOLOGY OF LAMBS INFECTED WITH MIXED GASTROINTESTINAL HELMINTIC PARASITES.

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ABSTRACT

Development of resistance to anti-helminthics by gastrointestinal parasites calls for an alternative method of their control. Nutritional manipulation has been proposed as one way of increasing resistance of host animals to gastrointestinal parasites. A 3x2 factorial experiment using 30 lambs aged between 5-7 months was carried out to investigate the effect of plane of nutrition on lambs' response to gastrointestinal parasite infestation. Half of the lambs were dosed weekly with 2500 infective larvae for five consecutive weeks (I_1) while the other group was un-infested (I_0). Each group was either supplemented with concentrate at two levels or not supplemented (S_0). The supplement groups received either 100g (S_1) or 200g (S_2) of concentrate containing 175g crude protein (CP)/kg dry matter. All animals were fed a basal diet of hay ad libitum. Crude protein digestibility was measured before dosing, at week 4 of dosing and 4 weeks post dosing. Faecal samples for egg count were collected immediately before dosing and thereafter every two weeks up to 15 weeks starting from second week. Similarly blood samples for haematological studies were collected before dosing and every two weeks starting from week 4 of infestation up to 15 weeks. Faecal egg output was observed from week 3 of infestation and persisted to the end of experimentation. Supplementation tended to decrease faecal egg count. There was time dependent effect of parasites on packed cell volume, haemoglobin, albumin, and total serum protein. Pre infestation values were higher ($P<0.05$) than infestation and post dosing values. Regardless of infection supplemented lambs had higher ($P<0.001$) haematological values than the un-supplemented group although there was no clear trend between the two levels of supplementation. Supplementation had no effect on crude protein digestibility. Infestation with mixed parasites significantly ($P<0.01$) reduced all haematological parameters and reduced significantly ($P<0.001$) the digestibility of crude protein. There was no significant interaction between nutritional supplementation and parasitic infection for most of the parameters measured although the supplemented group performed slightly better than the un-supplemented. It was concluded that nutritional manipulation can reduce severity of mixed parasitic infection but more work is required to determine the appropriate level of supplementation.

INTRODUCTION

Productivity of small ruminants in Tanzania is greatly reduced by poor nutrition and gastrointestinal nematodes. Gastrointestinal nematodes cause considerable economic losses through mortality, reduced growth rates, inefficient feed utilisation and reduced fertility. Such infection predisposes the animal to other diseases (Kelly and Hall, 1979; Sykes, 1994). Use of anthelmintics with greater than 95% effectiveness against parasite burdens has allowed control of parasite populations (Sykes et al 1992). However, it has been

observed that the target parasite populations have shown genetic variation in resistance to anthelmintic and selection towards resistant genotypes (Sykes et al 1992). Anthelmintic resistance is a worldwide problem and once acquired by parasite strain it is irreversible (Kelly and Hall 1979). In Tanzania Kassuku and Tibaijuka (1987) have reported anthelmintic resistance. Consequently, farmers have to use higher dosage or more frequent prophylactic routines, both of which are expensive alternatives.

Recently, there have been new approaches to helminth control strategies, which do not depend on chemotherapy. Nutritional intervention has been proposed as one of such strategies. (Gray, 1995). It has been shown that animals maintained on a good plane of nutrition are less affected by parasitic gastrointestinal nematode than poorly nourished animals (Gray, 1995, Coop and Holmes, 1996). Nutritional status of ruminants and protein nutrition in particular is an important factor influencing the susceptibility to infection of gastrointestinal parasites (Abbot and Holmes 1990). This influence is said to be associated with contemporary effect of nutritional modifications on the development of worms or some development of immunity by the host (Gray, 1995). Conversely, properly fed animals are better able to replenish blood constituents, which may be removed by blood sucking nematodes. During helminth infection there is a marked protein loss by the animal caused by leakage of plasma protein into gastrointestinal tract, increased cell turnover and mucus production and as a whole blood in case of blood ingesting worms (Coop, 1995).

There is paucity of information on the effect of level of concentrate supplementation on the response of animals to parasitic infections. The present study was therefore conducted to assess the effect of nutritional status on the patho-physiology of lambs infected with mixed gastrointestinal helmintic parasites.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was laid out in a 3x2 completely randomized factorial design using 30 weaned lambs. The treatments were two levels of parasite infection and three levels of supplementation making 6 treatments as shown below:
No supplement, no infection (S_0 i_0), T1
No supplement, plus infection (S_0, i_1), T2
Supplement (100 g), no infection (S_1, i_0), T3
Supplement (100 g), plus infection (S_1 i_1), T4
Supplement (200g), no infection (S_2, i_0), T5
Supplement (200g), no infection (S_2, i_1), T6

Experimental Animals and Management

Thirty (30) Black Head Persian lambs both males and females were weighed for two consecutive days to get the initial weights and randomly allocated to six treatments of five animals each. The animals were housed in individual pens with raised wooden slatted floor. Each pen was equipped with hay feeder, drinker and concentrate feeder for those supplemented. The animals were de-wormed using Milsan^R (1.5%w/v Levamisole + Oxyclosamide) before the start of experiment and when the faecal egg count exceeded

5000epg. The animals were introduced to experimental feed for the preliminary study period of three weeks.

Experimental Diet and Feeding

All the animals were fed a basal diet of hay (mixture of *Chloris gayana* and *Panicum maximum*), which was chopped and sprayed with urea solution at a rate of 20g urea in 600 ml of water per kg DM of hay. It was also sprinkled with molasses at a rate of 50g/kg DM in order to improve the quality and feed intake. The concentrate was composed of cotton seed cake (CSC), sunflower seed cake (SSC) and maize bran (MB) to contain 180g crude protein (CP) per kg DM. Common salt and mineral supplement (maclick) were added to the concentrate at a rate of 0.4% an 1% respectively. Mineral block was provided to animals, which did not receive concentrate supplement. The animals were fed the basal diet *ad libitum* at 9.00h and 14.00. The concentrate was fed only during morning feeding. Orts from each animal were collected and weighed before a new meal was given and samples were taken for DM determination. Clean drinking water was provided *ad libitum.*

Experimental Infestation of Animals

Infective larvae were obtained from a culture of faeces obtained from three infected sheep. The larvae were harvested, identified and the percentage distributions of larvae were determined. The larvae consisted of 53.3% H. *contortus*, 28.3% *O.columbianum*, 13.3% *T. colubriformis* and 5% strongiloides. They were stored at 4^0 C until used. A three weeks pre-infection measurement of digestibility, blood parameters and faecal worm egg count preceded the dosing of animals with infective larvae. The larvae concentration was made to 2500 larvae per ml and was kept at room temp for 8h before they were drenched to the animals. The animals were drenched with 2500 larvae weekly for 5 consecutive weeks.

Faecal Collection and Worm Egg Counting

Faecal samples for worm egg count were collected directly from the rectum of each animal before administering of larvae and subsequently every two weeks up to 15 weeks. The modified McMaster technique (MAFF, 1986) was used in determining worm egg from fresh faeces.

Blood Collection for Haematological Study

Blood samples were collected from all animals before dosing and thereafter fortnightly throughout the experimental period starting from the 4^{th} week after dosing. Blood was obtained by venipuncture of jugular vein into evacuated glass tubes with EDTA and plain tubes with separation gel and clot activator. Blood from EDTA tubes were used for measurement of packed cell volume (PVC) and haemoglobin (HB) concentration by micro-haematocrit and cyanmethemoglobin methods, respectively. Blood from the plain tubes were centrifuged, serum collected and stored frozen until analysed for albumin, and total protein by bromocresal green and biuret reaction, respectively (MAFF, 1978).

Crude Protein Digestibility

Digestibility study was carried out at three different periods, that is pre-dosing period, 4^{th} week of infection and 4 weeks after the last dosing (post dosing period) to investigate the

effect of parasitism on protein digestion. Three male lambs from each treatment were used for digestibility studies by total collection of faeces for seven consecutive days. During this period the animals were fitted with harness and collecting bags, which were lined with polythene bags. The bags were fitted in the morning and the faeces collected the following morning and weighed. After thorough mixing, 20% of the fresh faeces was put into air-tight plastic bags and stored in a deep freezer at -5^0C until analysed for nitrogen according to AOAC (1990).

Data Analysis

Crude protein digestibility and haematological parameters were statistically analysed according to General linear Model (GLM) procedure of the statistical analysis system (SAS,1990) for factorial design. Data on faecal egg count were analysed after logarithmic transformation.

RESULTS

Feed supplementation had a significant effect on all haematological parameters measured. The supplemented groups had higher ($P<0.01$) mean PCV values than the un-supplemented group. However, the PCV values were shown to decline as the experiment progressed such that the pre-infection values were higher for all treatments than during infection and post dosing (Table 1). Significant decline ($P<0.01$) in PCV for the infected group than the un-infected group was observed from week 4 of infection up to the end of the experiment. The mean PCV values for the infected group was lower ($P<0.01$) than the un-infected group (29%vs 25% Table 1). Similarly the mean PCV values was higher at pre-infection than at infection and post- dosing periods.

Animals offered higher level of nutrition had higher ($P<0.001$) haemoglobin concentration than the un-supplemented group. The infected animals showed lower mean haemoglobin concentration than the un-infected group. Decline in Haemoglobin with time was observed for both infected and un-infected where lower values were observed at four weeks of infection followed by a slight recovery during post infection period.

Table 1 Least square means for main effects of supplementation, infection and period on Crude protein digestibility and haematological parameters.

Factors	Parameters				
	Total protein (g/l)	Albumin(g/l)	Haemoglobin (g/l)	Packed cell volume (%)	CP digestibility
Supplementation level					
S_0	44.9^b	28.7^b	78.8^c	24.5^c	0.56
S_1	51.5^a	30.6^a	87.9^b	27.5^b	0.58
S_2	49.7^a	31.2^a	92.2^a	29.4^a	0.60
Significance	***	**	***	***	NS
Infection level					
I_0	51.0	31.6	92.0	29.0	0.62
I_1	46.4	28.7	80.5	25.0	0.55
Significance	**	***	***	**	***
Experimental Period					
Pre-dosing	50.5	32.8^a	99.5^a	31.0^a	0.62^a
Infection	48.7	31.6^b	80.1^b	27.4^b	0.60^a
Post-dosing	48.9	28.8^c	84.1^b	25.8^c	0.53^b
Significance	Ns	*	*	*	**

*=P <0.05; **= P< 0.01; *** = P<0.001; ns = non significant

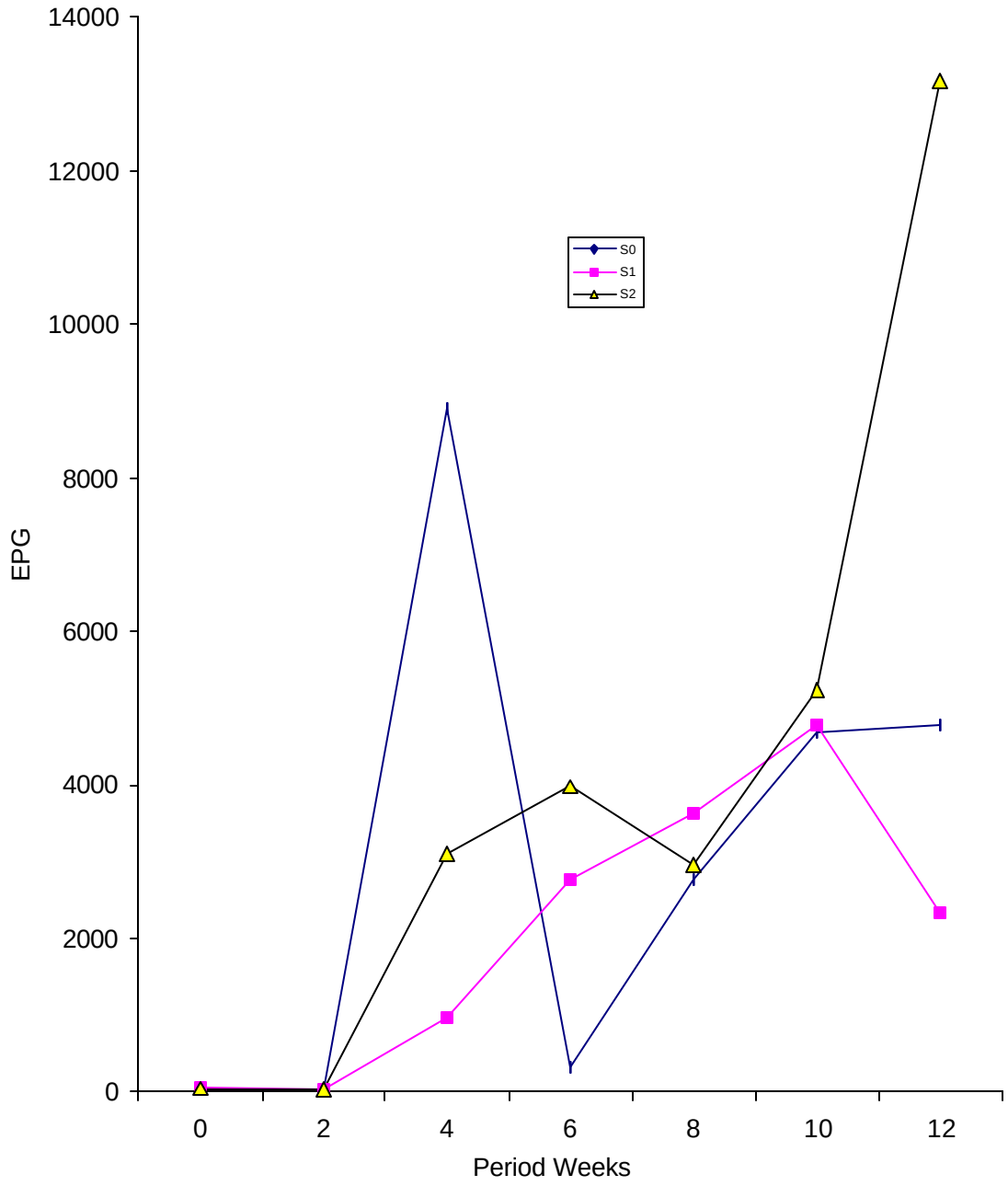

Figure 1: Effect of plane of Nutrition and infection on egg counts per gram (EPG)

Serum albumin was higher ($P< 0.01$) for the supplemented groups and ($P<0.001$) for the un-infected than the infected group. Albumin concentration during the pre-infection period was higher ($P<0.05$) than during infection and post dosing periods. The main effects of supplement and infection on serum total protein are shown in Table 1. Supplementation significantly ($P<0.05$) increased the total protein. S_1 group had higher total serum protein than S_2. The infected group had lower serum total protein than the un-infected group. Period had no significant effect on serum total protein although it was slightly higher during the pre-infection period.

The results on digestibility of CP shows that supplementation had no effect while infection lowered ($P<0.001$) the digestibility. The CP digestibility post- dosing was significantly ($P<0.01$) lower than during pre-infection and infection period.

The results on faecal egg excretion (Figure 1) show that the supplemented groups excreted fewer eggs in the first four weeks whilst the un-supplemented group excreted higher worm eggs which necessitated administration of antihelmintics. With time the medium level supplementation seemed to perform better than the high level supplementation in terms of faecal egg excretion.

DISCUSSION

The significant depression in apparent digestibility of crude protein of infected group as compared to the control group in the present study agrees with the findings of Sykes and Coop (1977) and Steel *et al.* (1980). However, it contradicts the earlier findings of Reveron *et al.* (1974) and Sykes and Coop (1976). A reduction in protein digestibility could result from loss of differentiation and function of acid secreting parietal cells and pepsinogen secreting zymogen cells as a result of damage to the abomasum. The increased faecal nitrogen in infected group could arise from increased endogenous loss of plasma protein, which has been reported by Kimambo *et al.* (1988) in sheep. The significant differences observed in digestibility of CP at different periods of experiment is the result of cumulative effect of the parasites on proper functioning of the gut. Likewise the decline in haematological parameters in infected group could be a result of blood sucking H. *contortus* (which constitute 53.3% of the infective larvae) compounded by reduced feed intake among the infected animals. Abbott and Holmes (1990), Blackburn et al (1991) and Shaw et al. (1995) have reported similar findings in growing goats and lambs. This indicates that supplementation increased the availability of nitrogen from the supplement for the synthesis of haematological parameters. The slower increase in faecal egg count for the supplemented groups in the first 10 weeks (Figure1) might indicate that the animals inhibited larvae development leading to fewer numbers of parasites. The observed decline in faecal egg count observed for the un-supplemented group was due to administration of anthelmitic to this group on the fourth week of dosing due to very high egg counts. The lower faecal egg excretion by the animals supplemented at medium level than those supplemented at higher level is difficult to explain. However it may imply a kind of interaction which favour parasite survival at higher level of supplementation as shown by rapid rise in faecal egg count towards the end of experiment. Similar findings have been reported by Gimbi (2000) in goats (unpublished report).

CONCLUSION

It can be concluded that although supplementation did have a positive influence (especially the medium level) on the performance of parasitised lambs, it was not sufficient to reduce completely the negative effects of parasitism. Therefore more work on proper level of supplementation should be undertaken.

ACKNOWLEDGEMENT

The Authors wish to thank NORAD and DANIDA through ENRECA for financial support.

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THE POTENTIAL FOR NITROGEN RECYCLING INTO THE RUMEN OF SHEEP FED FORMALDEHYDE TREATED AND UNTREATED COTTON SEED CAKE AS PROTEIN SUPPLEMENT TO POOR QUALITY HAY.

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ABSTRACT

Dry matter intake, digestibility of poor quality hay and nitrogen utilisation were measured in five rams fed 5 treatment rations in a 5 x 5 Latin square design in a study to evaluate the effect of formaldehyde treatment of protein supplement on nutrient utilisation and nitrogen recycling. The basal diet (T1) was composed of poor quality hay enriched with urea to attain a crude protein (CP) value of 7%. The other treatment diets (T2-T5) were composed of poor quality hay supplemented either with formaldehyde treated or untreated cottonseed cakes (CSC) to raise the diet CP value to 10% and 13%. Formaldehyde treated CSC was used in diet T4 and T5 while for T2 and T3 untreated CSC was used. The CSC was milled and mixed with hay to avoid selection of CSC and the diet was offered adlibitum. Significant increase ($P<0.05$) of DM intake (46.1 to 54.4 and 53.2 g/kg$w^{0.75}$) was observed when the CP content of the basal diet was increased from 7% in T1 to 10% (T2) and 13% (T3) CP using untreated CSC. There was no significant ($P>0.05$) difference in DM intake between T2 and T3. Increasing the CP content of the diet from 7% to 10% using formaldehyde treated CSC had no effect on the DM intake. However raising the CP to 13% using formaldehyde treated CSC gave a significant increase ($P<0.05$) in the DM intake from 46.1 to 53.3 g/kg$w^{0.75}$. Dry matter digestibility was significantly ($P<0.05$) higher for T3 than the other treatments (54.1% 57.8%, 67.8%, 53.1% and 57.8% for T1, T2, T3, T4, and T5 respectively). At the same level of CP content in the diet, treatment with formaldehyde reduced the dry matter digestibility. Urinary nitrogen (N), urinary urea-N and plasma urea-N were significantly higher ($P<0.05$) in treatments with untreated than treated CSC. Formaldehyde treatment lowered significantly ($P<0.05$) the rate of protein degradation in the rumen, however similar intestinal digestion of protein was observed between formaldehyde treated and untreated cotton seed cakes. It is concluded that feeding formaldehyde treated CSC at low level of protein inclusion, reduces feed utilisation because of the reduction of the amount of nitrogen available to the rumen microbes and that nitrogen recycling may not suffice N requirements of rumen microbes.

Keywords: Nitrogen recycling, Formaldehyde, Cotton seed cake, protein supplement, Rumen, Sheep, Poor quality hay

INTRODUCTION

Strategic supplementation with rumen degradable protein has been advocated as means of improving the utilisation of low quality roughage (Aganga *et al*, 1983). On the other hand

ruminants fed diets low in rumen degradable protein have the ability to recycle blood urea into the rumen for microbial protein synthesis and thus reducing urinary urea nitrogen excretion and rumen degradable protein requirement (Alawa, 1991). Nitrogen is recycled into the alimentary canal of ruminants via saliva (McDonald *et al*., 1988) or by diffusion from blood through the rumen wall (Kennedy and Milligan, 1978; Ørskov, 1982). The positive effects of recycled nitrogen on ammonia concentration, and microbial numbers in the rumino-reticular digesta is well documented (Van Soest, 1994) although the extent and rate of nitrogen recycling is variable due to several factors (Kennedy and Milligan 1980).

The ability to recycle blood urea into the digestive system under low nitrogen intake is dependent on the relative ability of animals to reduce the amount of urinary nitrogen excretion (Benlamlih and Pomyers, 1989). Decrease in urea glomerular filtration rate and increase in renal re-absorption of filtered urea brings about reduction in the amount of urinary nitrogen excretion. At the same nitrogen intake goats recycled more nitrogen into the rumen than sheep (Dominique *et al.* 1991) and buffalo calves recycled more than cattle calves (Dhiman and Arora, 1990) indicating species variation.

There is an inverse relationship between blood urea nitrogen transferred into the rumen of different ruminant species and level of nitrogen intake (Kennedy and Milligan, 1980; Bunting *et al.*, 1987; 1989; Obara and Shimbayashi, 1988). The resistance of different protein sources to microbial attack in the rumen influences the amount of blood urea nitrogen recycled into the rumen. Higher proportion is observed in animals fed protein of low rumen degradability (Sultan *et al.* 1992, and Faichney, *et al* 1994).

There is therefore a scope for improving protein utilisation if degradability of highly rumen degradable proteins is reduced before they are fed to ruminants. Thus, efforts to increase the understanding of proper use of protein supplements and how to improve the utilisation of recycled N are worthwhile to consider.

Proper utilisation of recycled N is important because feeds high in protein content are expensive in the developing countries whereas in developed countries there is a need to minimise environmental pollution caused by high amount of N excreted in faeces and urine. The objective of the present study was to evaluate the effect of feeding diets containing different levels of protein of different rumen degradability on the utilisation of poor quality roughage in terms of DM intake, digestibility and nitrogen excretion.

MATERIALS AND METHODS

Experimental design and Treatments

The experiment was laid out in a 5 X 5 Latin Square design involving 5 rams, 5 treatments and 5 periods. The rams were fed each of the treatment diets for 18 days in each period, which consisted of 10 days for adaptation and 8 days for data collection.

Experimental Animals and their Management

Five mature (Black Head Persian) rams with initial body weights ranging from 33 to 40 kg were used. The animals, which were kept in metabolic cages and fed individually, were weighed at the beginning and end of each period. De-worming of the animals was done before the