THE RESPONSE OF *FASCIOLA GIGANTICA* INFECTED SHEEP TO DIETARY NITROGEN

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SUMMARY

Chronic Fasciola gigantica infections were established in lambs maintained on diets differing in their protein and nitrogen content. The course of the disease and their production parameters were compared with that of similarly maintained uninfected controls. Parasitological, haematological, serological, biochemical and post-mortem data indicated light F. gigantica infections were successfully established in the experimentally infected sheep. Nutritional and production parameters indicated little difference in the growth rates of infected and control sheep maintained on medium (14%) protein diets and that a proportion of the dietary nitrogen (2.4%) could be derived from urea without detrimental effect on production parameters. In contrast infected sheep maintained on either high (>19%) or low (7%) protein diets did not grow as well as their respective controls and the former group displayed a degree of inappetance. It is therefore suggested that a diet containing approximately 14% protein may help alleviate the negative production effects often associated with fasciolosis. This level is close to that generally recommended for ruminant diets but since many ruminants in Nepal are maintained on diets of a lower protein content, feed supplementation to increase the overall protein content of the diet may be a suitable option. Similarly diets with too high a protein content (>19%) should be avoided in areas endemic for fasciolosis.

INTRODUCTION

Fasciolosis is well recognised as a serious constraint to animal production in Nepal, accounting for a losses of 20% and 14% to buffalo and goat producers alone (Mahato, 1993). The extent of the problem, as recognised by farmers, is reflected in the high percentage of the total drug sales (60% Mahato, pers. comm.) that are devoted to drugs for the treatment of fasciolosis. Consequently, a high priority is placed on the control of fasciolosis by the Nepalese Government. There is relatively little quantitative information known about the negative effects of fasciolosis, particularly for *F. gigantica* (Roberts and Suhardono, 1996) but studies into the epidemiological basis for the control of fasciolosis in Nepal have been conducted (Mahato, Harrison and Hammond, 1997). While strategic and symptomatic drug treatment are an option for control (Shrestha and Joshi, 1997), farmers often cannot afford to treat their animals with expensive drugs, even if they are available locally. A more economic and feasible management strategy may be to supplement the diet of the animals with locally available feeds to improve their nutritional status, as was found to help in the closely related parasite *Fasciola hepatica* (Berry and Dargie, 1976; Dargie, Berry and Parkhins, 1979). This experiment was designed to test the hypothesis that the amount and source of dietary nitrogen can influence the progress of the infection, inappetance and rate of growth in *F. gigantica* infected sheep. The effect of sourcing some of the dietary nitrogen from urea was investigated because of the interest in Nepal of using urea molasses blocks as a feed supplement.

MATERIALS AND METHODS:

Experimental design:

A total of 48 Suffolk Cross lambs were split into four diet groups and maintained in individual pens. The sheep were fed *ad libitum* (Table 1). The composition of the diets and analysis of the diets, which were made up at the Scottish Agricultural College, were as detailed in Tables 2 and 3. The diets were prepared in batches of 2000Kg. The sheep were also give 150-200g of hay every day to ensure that rumination was maintained. The sheep were infected with *Fasciola gigantica* (2 metacercariae/Kg at week 15) and examined at *post-mortem* 24 weeks later.

Table 1 Experimental design - numbers of sheep in each of the dietary groups.

Diet Group (crude protein)	Infected with *Fasciola gigantica*	Uninfected Controls
High protein (20%)	6	6
Medium Protein (14%)	6	6
Medium Protein plus Urea (14% includes 2.45% urea)	6	6
Low Protein (7%)	6	6

Table 2 Composition of the diet batches for the four different dietary groups.

Diet Group Protein/ % content	High	Medium	Medium plus urea	Low
Barley	20.6667	19.5000	42.8500	40.8550
Oatfeed	19.3333	22.5000	17.0000	21.5000
Citrus pulp	29.9667	29.8750	30.0000	30.0000
Hi-Pro Soya	20.6667	20.5000		*
Molasses (CMS20)	5.0000	5.0000	5.0000	5.0000
Salt	0.6800	0.6800	0.5350	0.68000
Dicalphos	0.4800	0.4900	0.7900	0.7900
Limestone flour	1.0067	1.2550	0.9950	0.9750
Scotmin ewe/lamb	0.2000	0.2000	0.2000	0.2000
Sodium sulphate	*	*	0.1800	*
Urea	2.000	*	2.4500	*
Total	100	100	100	100

Monitoring:

The sheep were monitored regularly throughout the entire experimental period and the following parameters determined:

Diets: The feed batches were monitored regularly during storage to ensure consistency of nitrogen content, neutral detergent fibre, acid detergent fibre and organic matter.

Intakes: The feed intakes/refusals were monitored following every feed so that the total dietary intake could be determined.

Parasitological: Faecal samples were collected every week and the numbers of fluke eggs per gram calculated using standard procedures,

Haematology: Blood samples were collected weekly in order that total red and white blood cell counts could be taken. Differential cell counts were noted. The haemoglobin level in the blood and the packed cell volumes were determined so that, along with the total red blood bell counts, the Mean Corpuscular Volume, Mean Cell Haemoglobin and Mean Cell Haemoglobin Concentration could be calculated.

Table 3 Feed analysis for the four different dietary groups.

Diet Group Protein/ Analysis (Vol 100.0)	High	Medium	Medium plus urea	Low
Dry Matter	872.9733	871.6500	873.2950	871.3885
Crude Protein	199.4793	140.9575	140.5550	69.4600
ERDP 5%	145.7237	98.6963	108.6490	51.3461
ERDP 8%	133.9983	87.0763	105.1365	47.8884
DUP 5%	28.91	28.7590	9.850	9.8965
DUP 8%	39.2610	39.0263	12.5833	12.5640
ME RUM	9.5086	9.5151	9.4981	9.5111
FME	9.0119	9.0075	9.0495	9.0461
FIBRE	101.7867	108.6600	96.5110	106.3933
MADF	161.8767	172.2975	147.7675	162.8703
NDF	255.1817	272.3363	250.4590	274.8877
STARCH	125.9127	123.4225	228.0750	222.9345
SUGAR	102.4233	101.9175	86.9990	86.7197
STA+SUG	229.0284	226.0413	315.5455	310.1457
ASH	69.7791	73.2862	64.2909	65.2171
CALCIUM	9.9972	10.9914	10.0063	9.9892
PHOSPHORUS	3.5040	3.5173	3.5018	3.4994
MAGNESIUM	1.2325	1.2169	0.9370	0.9193
SODIUM	2.9912	3.0040	3.0004	3.0012
POTASSIUM	10.2588	10.3341	6.1276	6.2893
SULPHUR	1.7447	1.7545	1.5042	1.1203
COBALT	0.8701	0.8694	0.8479	0.8473
COPPER	6.6192	6.6689	4.3369	4.4351
IODINE	4.8556	4.8554	4.8350	4.850
MANGANESE	53.6163	54.0213	47.7705	48.4112
SELENIUM	0.2503	0.2492	0.1971	0.1963
ZINC	56.7703	57.0088	51.7410	52.1223
VIT A (K)	8.0000	8.0000	8.0000	8.0000
VIT D	1999.9980	1999.998	1999.998	1999.998

Biochemistry: Serum samples were collected weekly so that the serum levels of the enzymes, γ-glutamyl transferase, glucose-6-phosphate dehydrogenase, β-hydroxybutarate and the serum glucose, nitrogen, protein and albumin levels determined, all by standard procedure using kits prepared by Randox Ltd.

Serology: A routine indirect antibody detection ELISA was employed in order to determine the antibody responses of the sheep to *F. gigantica* infection. Excretory/secretory products collected from adult flukes maintained in culture for 24 hours was used as the antigen, diluted to optimum concentration, as determined by titration. Following addition of the test serum diluted to an appropriate concentration, the presence of anti-parasite antibody was detected by the addition of commercial rabbit anti-sheep (H+L chain) serum/ horseradish peroxidase conjugate, followed by tetramethylbenzidine substrate (Sigma Ltd). The colour reaction was stopped using 0.2M H_2SO_4 and the optical density of the reaction product measured at 450nm.

Physiological: The sheep were weighed twice weekly so that their weight gain and metabolic weights could be determined.

Post-mortem: At the time of slaughter, the carcass condition was recorded. The dressed out carcass weights were determined and the degree of liver pathology noted. The number of flukes present in the

livers of the infected animals was calculated by slicing the livers carefully to look for the flukes. Bile was collected and examined to determine whether there were any fluke eggs present.

RESULTS

Feed analysis indicated that the omposition remained constant throughout the experimental period. None of the sheep in the control groups gave any indication of being infected with *F. gigantica* by any of the monitoring parameters. All the indications were that the infections established in the exposed animals were mild with small numbers of flukes (in the range 1-11) being recovered from the infected groups, but there was no statistical difference in the number of flukes recovered form each dietary group. This conclusion was confirmed by the haematological and biochemical data. The serological results also indicated infection in the exposed group (Fig 1).

There was no difference in the weight gain following infection in either of the medium protein (14% crude protein groups) whether the protein was derived totally from crude protein (medium protein group) or partly derived from urea (2.45%), medium protein including urea group.

Unfortunately during the course of the experiment two of the sheep in the high protein (20% crude protein) group died. This group also exhibited a degree of inappetance as compared to the corresponding uninfected controls.

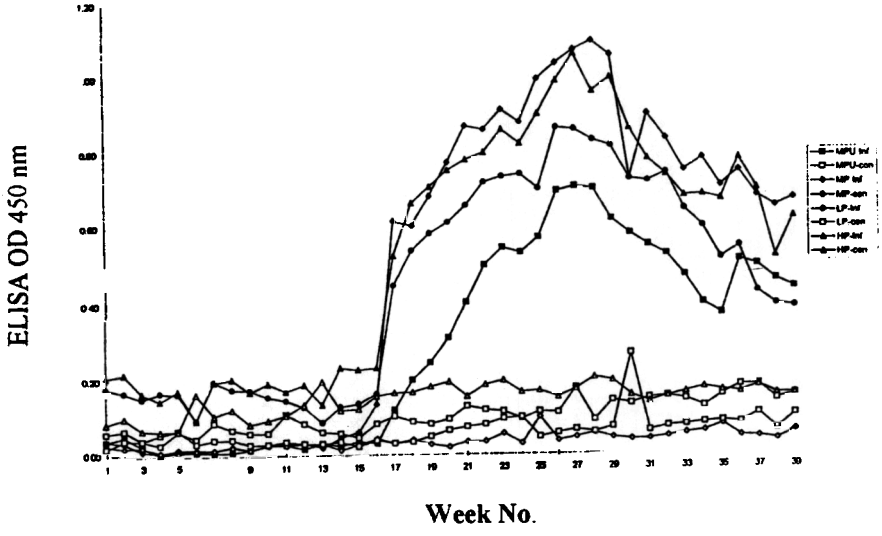

Figure 1: The antibody responses as measured by enzyme-linked immunosorbent (ELISA) of *Fasciola gigantica* infected and control sheep maintained on different diets medium protein plus urea (MPU) medium protein (MP) high protein (HP) or low protein (LP). The sheep were infected with metacercariae on week 15.

Regression analysis indicated a significant difference between infected and control sheep in both the high and low protein groups. The infected sheep grew less well than the uninfected controls over the entire post-infection monitoring period, although this was not the case in the pre-infection period. This effect was slightly more pronounced in the high protein infected group (Fig 2).

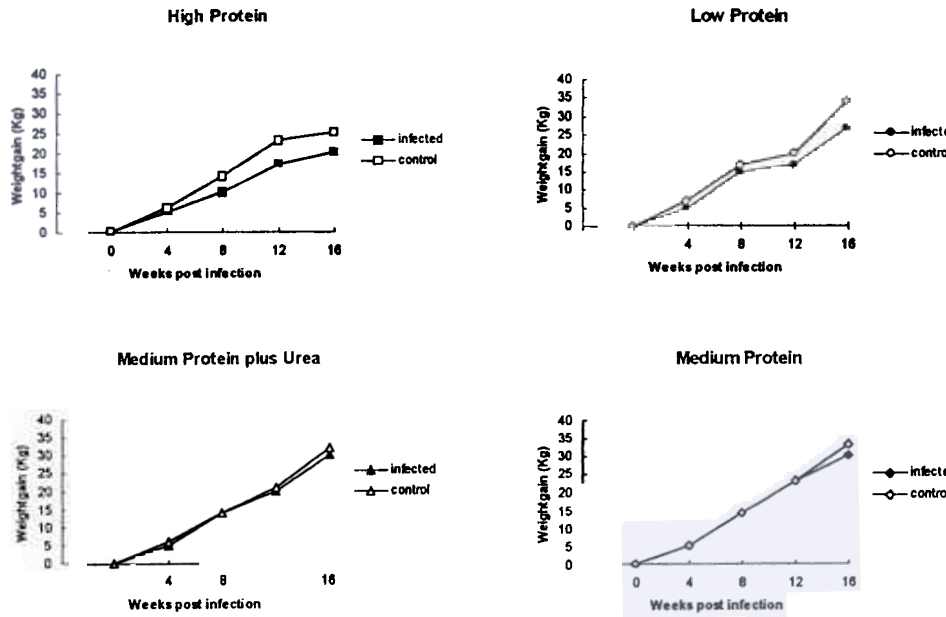

Figure 2: The weight gain following infection of *Fasciola gigantica* infected and control sheep maintained of different diets either high protein, medium protein, medium protein including urea or low protein.

DISCUSSION

Flukes specifically damage the livers of infected hosts. The liver plays a pivotal role in the physiology of the body, as it is responsible for a large proportion of the body's amino acid metabolism along with other important roles. At the same time, the liver has remarkable functional redundancy and, unlike most other organs in mammals is able to regenerate functional tissue after physical or chemical injury. Although only a few aspects of liver function have been directly examined in fluke-infected hosts, significant disturbances have been detected, even when only small areas are obviously damaged. Thus even small numbers of flukes have been associated with changes. It seems likely that the availability of amino acids for protein synthesis would be an important factor in determining the extent to which the liver can compensate for the damage caused by the flukes (Behm and Sangster, 1998).

This study indicate an appropriate level of dietary protein for *F. gigantica* infected sheep would appear to be c14% crude protein and that some of the dietary nitrogen can be derived from urea with minimal negative affect. However, the indications are that deviating too far from this level >19% and <8% crude protein has a deleterious effect.

Unfortunately many ruminants in Nepal are thought to be maintained on diets which have less that 14% protein. The suggestion therefore is to investigate the effect of using locally available feed supplementation to determine whether raising the protein content of the diet in ruminants managed

under Nepalese conditions would help to alleviate the negative production effects of fasciolosis. Such supplementation may form a useful alternative to drug treatment (either strategic or symptomatic), which currently is the main control method available. Unfortunately many Nepalese farmers find this method difficult to apply either because of cost or lack of easy availability of the appropriate drugs. If suitable, such food supplementation may form a useful adjunct to drug treatment regimens or even avoid potential problems with drug treatment such as drug residues in meat and milk and drug resistance.

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