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PATHOPHYSIOLOGY OF GASTROINTESTINAL NEMATODE INFECTIONS IN THE RUMINANT

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Summary

Recent advances in the understanding of the physiological and biochemical consequences-of gastrointestinal nematode infections in ruminants are examined, particularly in relation to the nitrogen economy of the host and effects on animal productivity. Evidence is presented which suggests that, although there may be an apparent reduction in the net availability of nitrogen from the gut of infected animals, this is probably due to an increased secretion of endogenous nitrogen rather than decreased digestion and absorption of dietary nitrogen. Increased leakage of plasma proteins into the gut is a feature common to many nematode infections of ruminants and is considered an important source of increased endogenous nitrogen secretion. This leakage also results in an increased albumin turnover, which, in laboratory animals, is associated with an elevated hepatic protein synthesis. Together with inappetance, these changes are probably responsible for decreased synthesis of meat and wool protein through their influence on the availability of amino nitrogen at the tissue level.

I. INTRODUCTION

Infection by parasitic nematodes of the gastrointestinal tract of sheep and cattle is characteristically associated with impaired animal productivity. In calves and lambs the principal outward manifestation of disease is a reduced rate of liveweight gain; in severely affected animals diarrhoea and a marked loss of weight are frequently reported symptoms. In infected sheep substantial reductions in wool growth have been recorded. These overall effects are a feature of infection with most of the commonly occuring species of parasitic nematodes and appear to be independent of the site of infection, whether this be the abomasum, small or large intestine. These losses in productivity are commonly accompanied by inappetance but cannot be explained solely by reduced nutrient intake. For example, Bremner (1961) found that calves with large-intestinal infections of *Oesophagostomum radiatum* gained weight at a rate lower that that of worm-free calves which were offered similar amounts of feed to that consumed by parasitized animals. Depressed liveweight gain per unit of feed intake has also been reported in calves with abomasal infections of *Trichostrongylus axei* (Cauthen and Landram 1958), and in sheep with small-intestinal infections of *T.colubriformis* (Franklin, Gordon and MacGregor 1946; Barker 1973) or large-intestinal infections of *Oe.columbianum* (Bawden 1969). Similarly, clean wool production may be reduced by as much as 60% in sheep infected with *T.colubriformis* compared with worm-free animals on similar feed intakes (Carter, Franklin and Gordon 1946; Barger, Southcott and Williams 1973).

An important factor in the understanding and control of parasitic disease is the definition of the underlying physiological and biochemical disturbances which occur in the host and are manifested by impaired productive performance. These aspects have received increased attention over the last few years and Symons (1969)

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gave a comprehensive account of earlier studies in his review of the pathology of gastrointestinal parasitism. In the present paper particular attention is given to recent advances in the understanding of nitrogen metabolism in these infections. Three distinct but interrelated areas of investigation are examined, namely digestion and absorption, endogenous losses of nitrogen in the gut and protein metabolism.

II. DIGESTION AND ABSORPTION

In view of the inappetance and apparent depression in the efficiency of food utilization, which together with diarrhoea, are commonly described symptoms of gastrointestinal worm infection, it is not surprising that derangement of digestive function is often suggested as a major factor in the pathogenesis of parasitic disease. Attempts to test this hypothesis *in vivo* have, until recently, usually been made through studies of the apparent digestibility of dietary constituents by conventional balance trial techniques. Such measurements have generally demonstrated a depression of nitrogen (N) digestibility in sheep infected with *Ostertagia circumcincta* (Horak and Clark 1964), *T. axei* (Spedding 1954; Ross, Purcell and Todd 1970) and *T. colubriformis* (Franklin, Gordon and MacGregor 1946; Horak, Clark and Gray 1968; Reveron, Topps and Hunter 1970; Barger 1973) but conflicting results have been reported (see Symons 1969). Few studies have been made of feed digestibility in cattle infected with parasitic nematodes. The results of Ross, Todd and Purcell (1970) suggest that calves with abomasal infections of *T. axei* had lower digestibilities of protein, fat and fibre but, as in many other studies of this type, interpretation of their data is confounded by the marked inappetance of infected animals which may introduce large errors into the calculation of apparent digestibility when based on short-term collection periods.

To alleviate these effects and also to enable a differentiation to be made between effects attributable to anorexia and those attributable to the parasite *per se* some workers have made comparisons between infected and worm-free animals which were pair-fed. This procedure was used by Parkins, Holmes and Bremner (1973) in a study of nitrogen balance and 'feed digestibility of sheep infected with a single inoculum of 500,000 to 1,000,000 *O. circumcincta* larvae. At the higher level of infection, during the 7 to 21 day post-infection period both infected sheep and their pair-fed controls were in negative nitrogen balance. However, in infected animals nitrogen retention was significantly lower than in the pair-fed group; this was largely due to a higher urinary N excretion in the infected sheep rather than a higher faecal N output, although the apparent digestibility of N was reduced by the infection.

In reviewing the effects of gastrointestinal nematode infections on digestion and absorption, Symons (1969) emphasized the limited value of digestibility trials in determining changes in digestive function. Some assessment of overall\ effects can be made, but it is not possible to determine whether a depression in N digestibility is due to impaired digestion and absorption *per* se or to increased endogenous losses of N into the gut or both. Furthermore, results may be misleading in terms of assessing the availability of substrates for metabolic processes since it is not possible to partition digestive function in different regions of the alimentary tract. This is particularly important in the case of the nitrogenous component of the diet, since fermentative activity in the rumen may substantially alter the amount and composition of the amino acid component of ingesta which subsequently becomes available for absorption in the small intestine. Similarly microbial activity in the caecum may tend to mask any reduction in the digestion and absorption of nitrogen from the small intestine because of degradation of undigested protein passing from the small intestine.

To overcome some of these limitations, Symons and Jones (1970) used sheep prepared with cannulae in the abomasum and distal ileum to study N digestion and absorption in the small intestine of animals given 40,000 to 60,000 infective *T.colubriformis* larvae. In separate experiments an indigestible marker together with either casein or a ^{14}C labelled preparation of *Chlorella* protein was infused through the abomasal cannula and digesta samples taken several hours later from the ileum for analysis of N or ^{14}C relative to the concentration of marker. Both methods indicated that there was no impairment of digestion and absorption of protein in the small intestine. Similar results were obtained with rats and mice with small intestinal infections of *Nippostrongylus brasiliensis* and *Nematospiroides dubius* respectively when ^{14}C-chlorella protein was given as a test meal and its disappearance measured by radioactive analysis of ileal contents approximately 20 hours later. These results confirmed earlier studies of nippostrongylosis in the rat which indicated that, although digestion and absorption at the site of infection, that is the jejunum, may be depressed, it was compensated for by an increased rate of absorption from the distal ileum, such that overall digestion and absorption from the small intestine was unaffected (see Symons 1969, 1971). Symons and Jones (1970) concluded that the inferior productive performance of infected animals could not be ascribed to a failure of digestion or absorption in the small intestine.

The possibility that wool growth in sheep with intestinal trichostrongylosis was limited by malabsorption of sulphur-containing amino acids known to be essential for wool protein synthesis was examined by Barger, Southcott and Williams (1973). The wool growth response to a daily supplement of cysteine given either intravenously or intraduodenally was compared in infected and non-infected sheep which were pair-fed. Although the infection depressed wool growth by 18%, the wool growth response to cysteine supplementation was similar in both groups of animals and was not influenced by the route of administration. It was concluded that reduced wool growth induced by trichostrongylosis could not be attributed to malabsorption of cysteine.

In this laboratory, quantitative aspects of digestion at various sites in the gastrointestinal tract are being studied in sheep under normal dietary conditions as a first step in an assessment of the effects of nematode infections on the availability of substrates for metabolic processes. In normal sheep, approximately 60% of the digestible energy derived from the diet can be accounted for by volatile fatty acids (VFA) resulting from microbial fermentation of ingesta in the rumen (see e.g. Leng, Corbett and Brett 1968). Oxidative metabolism of the principal VFA produced in the rumen, namely acetate, propionate and butyrate, together may account for 70% of the total CO output of normal fed sheep (Annison *et al.* 1967). To determine whether impaired rumen function was a consequence of *T.colubriformis* infection, Steel (1972) made measurements of the rate of production of acetic acid and the movement of fluid in the rumen of lambs given 40,000 to 100,000 infective larvae. Compared with pair-fed controls acetate production was 30% lower in the infected animals; this depression was apparently not related to any change in rumen volume or the rate of movement of fluid from the rumen. These studies have recently been extended into a more detailed examination of the effects of trichostrongylosis on quantitative aspects of digestion at other sites of the gastrointestinal tract (Steel and Hogan, in preparation). Briefly, mature worm-free sheep were fitted with permanent cannulae into the rumen, abomasum and terminal ileum and offered a diet of ground and pelleted lucerne and wheaten hays containing, on a dry-matter basis, 91.3% organic matter (OM), 1.8% N and 55.5% cell-wall constituents (fibre). The flow of digesta from the stomach (i.e. rumen + reticulum + omasum + abomasum) and the small intestine was estimated by reference to the concentration in digesta samples from the abomasum and ileum of radioactive markers which were infused intraruminally. Appropriate chemical analyses of digesta samples enabled the flow of OM, N and fibre to be calculated (see Hogan 1973).

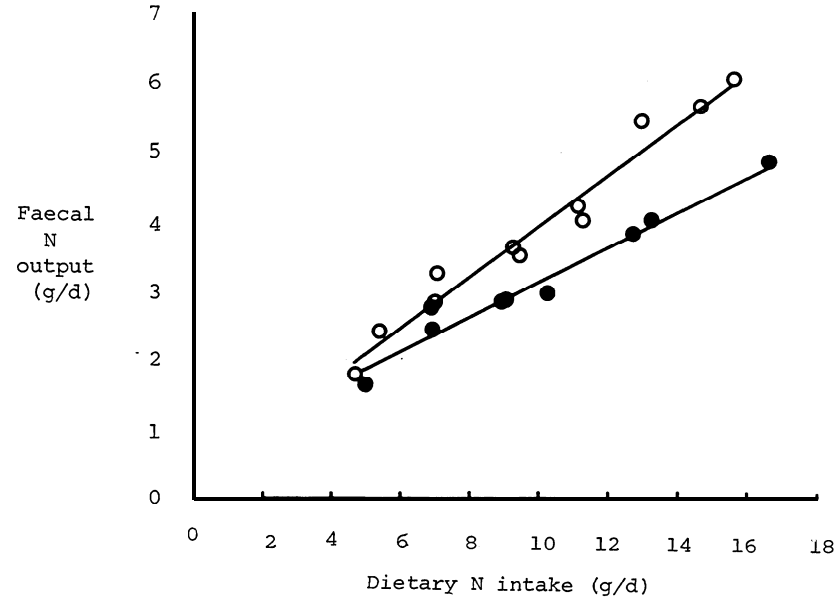

Fig. 1. Relationship between faecal N output and dietary N intake in sheep infected with *Trichostrongylus colubriformis* (O) and in worm-free sheep (●).

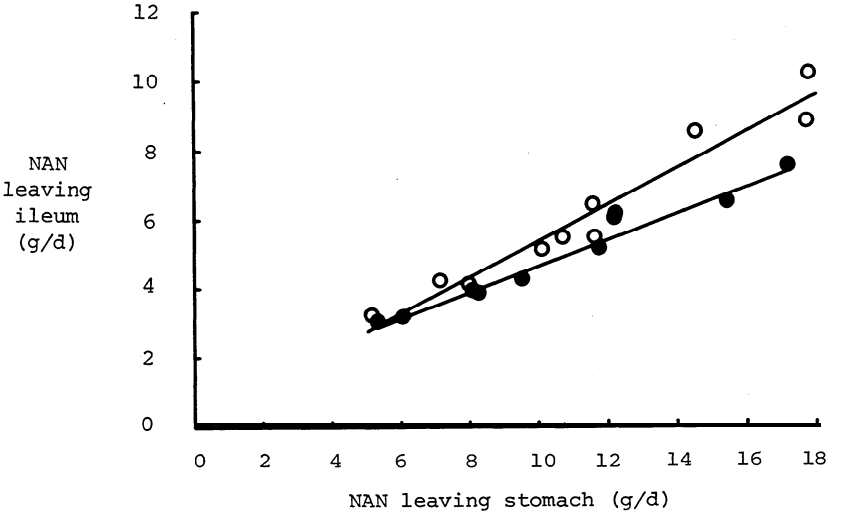

Fig. 2. Relationship between nitrogen in forms other than ammonia (NAN) leaving the ileum and NAN leaving the stomach in sheep infected with *Trichostrongylus colubriformis* (O) and in worm-free sheep (●).

Measurements were made in the same animals both prior to infection and approximately four to six weeks after the start of a twice-weekly inoculation with 5000 infective *T. colubriformis* larvae. Infected sheep were offered feed ad lib. and variable degrees of anorexia were observed; some control animals were given feed ad lib., others were given restricted amounts in order to achieve a range of feed intakes similar to that anticipated in the infected group. Intakes of OM, N and fibre for all sheep ranged from 260 to 830 g, 4.7 to 16.6 g and 160 to 520 g respectively.

Apparent digestion of OM, N and fibre were each significantly lower in infected animals than in the controls but, for the purposes of this communication, only data relating to N digestion are presented. In both infected and non-infected sheep there was a significant rectilinear relationship between faecal N output and dietary N -intake (Figure 1). The regression coefficient for the relationship in infected sheep was significantly greater ($P < 0.01$) than that for the worm-free group , indicating that differences in faecal N output become more marked in infected animals with increasing dietary N intake. Conversely, the results suggest that in animals which are severely affected by the parasite, that is exhibiting marked anorexia, there is little effect on apparent N digestibility other than that attributable to reduced feed intake.

The amounts of nitrogen in forms other than ammonia (NAN) entering and leaving the small intestine were rectilinearly correlated in both infected and worm-free sheep (Figure 2). However comparison of the two regressions showed that the slope of the relationship in infected sheep was significantly higher ($P < 0.05$) than in the uninfected group. Thus, there was apparently little difference between the amounts of NAN reaching the terminal ileum in inappetant, infected sheep and in worm-free animals on a similar feed intake. with increasing feed intake, the flow of NAN from the small intestine increased at a more rapid rate in infected sheep up to a maximum of 2 g/d higher than in the controls. Since a proportion of the NAN in ileal digesta is of endogenous origin, measurements of differences between the amounts of NAN entering and leaving the small intestine represent an apparent absorption, or net loss, of NAN in the small intestine. In normal sheep amino-acid nitrogen constitutes approximately 80% and 65% of NAN in abomasal and ileal digesta respectively (Hogan 1973). If it is assumed that these proportions apply to infected sheep also, then differences in net losses of NAN may be a reflection of the relative amounts of amino nitrogen absorbed from the tract in the two groups of sheep. However, this assumption is valid only if endogenous secretion of nitrogen into the gut is similar in both normal and infected sheep.

III. ENDOGENOUS SECRETION OF NITROGEN INTO THE GUT

The principal sources of endogenous nitrogen in the gastrointestinal tract are mucus, desquamated epithelium, urea, digestive enzymes and plasma protein (Phillipson 1971). Considerable quantities of nitrogen are secreted into the gut even in normal animals. In sheep fitted with intestinal loops, Kay (1969) estimated that secretion of nitrogen into the jejunum and ileum amounted to 1.5 g N/d with bile, pancreatic juice and duodenal secretion contributing a further 2 g N/d. Most of the soluble protein secreted into the small intestine consists of plasma protein, and estimates based on the catabolism of I^{131}-labelled albumin indicated that up to 8 g plasma albumin/d entered the small intestine in normal sheep (Campbell *et al.* 1961).

Infection of the small intestine of the rat with *N. brasiliensis* results in an increased turnover of jejunal epithelial cells (Symons 1965). Estimates of protein loss from the small intestine increased from 159 µg/min in normal rats to 554 µg/min in rats with nippostrongylosis; in both groups 8 to 15% of the protein was calculated to arise from intracellular protein, with the remainder arising from extracellular sources (Da Costa, Croft and Creamer 1971). Leakage

of plasma proteins into the gut at the site of infection has been clearly demonstrated in ostertagiosis and oesophagostomosis of ruminants (Jennings *et al.* 1968; Bremner 1969a) and hypoproteinaemia is a commonly reported feature of these and other nematode infections. Consideration of the quantitative aspects of plasma leakage is important in order to understand the aetiology of the hypoproteinaemia, and also to assess its effect on the overall nitrogen metabolism of the host.

Faecal excretion of radioactive chromium 51 (^{51}Cr) following intravenous injection of ^{51}Cr-labelled albumin or ^{51}Cr Cl$_3$, which binds to plasma proteins *in vivo,* has been widely used to estimate 'faecal plasma clearance', namely the volume of plasma which would have to leak into the gut to account for the daily excretion of faecal radioactivity. Faecal plasma clearance in young sheep two weeks after infection with a single inoculum of *O.circumcincta* averaged 87 ml/d compared to 29 ml/d in worm-free animals (Holmes and MacLean 1971). Mean serum albumin concentration in the two groups was 2.5 g/100 ml and 3.4 g/100 ml respectively, indicating that the loss of albumin alone into the gut in infected sheep was about 2.2 g/d compared with 1.0 g/d in the uninfected group. Similar studies with g-week-old lambs infected with *T.colubriformis* showed that an increased loss of plasma into the gut commenced 10 to 12d after infection, and coincided with the onset of inappetance, hypoproteinaemia and weight loss (Barker 1973). Comparisons were made between pair-fed animals and the estimated loss of total plasma protein into the gut of infected sheep was 6 to 12 g/d during periods of maximum plasma leakage compared to 1.8 g/d in the control group.

Clearly, in order to assess the nutritonal consequences of this endogenous protein loss it is necessary first to determine whether these proteins are digested and reabsorbed from the gut. In sheep infected with *O.circumcincta*, Parkins, Holmes and Bremner (1973) concluded that, since there was no significant increase in faecal nitrogen loss compared with pair-fed controls, nitrogen reabsorption was apparently unimpaired in this infection. However, as emphasised earlier, estimates based on faecal nitrogen excretion do not enable a differentiation to be made between digestion of plasma protein and absorption as amino-acid N in the small intestine, or degradation by microbial fermentation in the large intestine. with large intestinal infections the situation is more readily defined. In calves infected with *Oe.radiatum* Bremner (1969a, 1969b) showed that there was a substantial increase in the volume of plasma leaked into the caecum and colon. The loss of plasma protein exceeded that in pair-fed, worm-free animals by 40 g/d; a concurrent determination of nitrogen balance demonstrated that increased faecal nitrogen excretion in infected calves was equivalent to this protein leakage.

It seems likely therefore that in the studies of Steel and Hogan described above, increased amounts of plasma protein were secreted into the small intestine of sheep infected with *T.colubriformis*. The results shown in Figure 2 indicate that most of this nitrogen was digested and reabsorbed before the distal ileum in infected sheep which exhibited marked inappetance, so that net loss of NAN from the small intestine was apparently unaffected. However, in those animals in which appetite was only marginally affected by the infection, the capacity for complete digestion and reabsorption of endogenous protein was exceeded and net loss of NAN was apparently reduced. The increased flow of digesta through the small intestine in sheep on higher feed intakes supports this explanation which conforms with Kay's (1969) suggestion that, since protein digestion occurs throughout the length of the small intestine, there is little or no safety margin for the completion of digestion.

IV. PROTEIN METABOLISM

Alterations to the normal pattern of protein metabolism would be expected to result, either directly or indirectly, from the effects of parasitic infection on gastrointestinal nitrogen transactions described above. As mentioned previously, hypoalbuminaemia is a feature of most gastrointestinal nematode infections of ruminants, and particular attention has been given to the study of the kinetics of plasma albumin metabolism, principally in ostertagiasis of sheep and cattle (see Mulligan 1972). Albumin turnover rate, that is, the proportion of the albumin pool catabolized per unit time, increased markedly in both sheep and cattle following infection. However, as pointed out by Mulligan, because of the reduced size of the albumin pool in severely affected animals, the mass of protein degraded daily may not necessarily exceed that in a normal animal. Concurrent measurements in sheep following infection with *O. circumcincta* demonstrated a close association in time between increased albumin turnover and the occurrence of increased gastrointestinal plasma loss (Holmes and MacLean 1971). However it is not clear whether, in quantitative terms, elevated albumin turnover is due solely to an increase in exogenous catabolism in the gut. Holmes and MacLean (1971) presented evidence to suggest that endogenous catabolism of albuminmay also rise as a result of infection.

If steady state conditions prevail in the albumin pool, it is reasonable to assume that measures of albumin turnover reflect the rate of replacement or synthesis of albumin. The extent to which increased albumin turnover causes diversion of amino acids away from protein synthesis in tissues is not known. Rises in urinary nitrogen excretion and elevated plasma urea levels in sheep with ostertagiasis compared with pair-fed, uninfected animals (Parkins, Holmes and Bremner 1973) indicate a detrimental effect of infection on the nitrogen economy of the host, not accountable for by reduced feed intake alone. These changes may reflect the mobilization of tissue protein reserves to meet an increased amino nitrogen requirement for albumin synthesis.

To date, little information is available on the biochemical effects of ruminant nematode infections on amino acid incorporation into tissue protein at the cellular level. However, these aspects have recently been examined with small intestinal infections of laboratory animals. Symons and Jones (1971) measured the incorporation of ^{14}C-L-leucine into liver and muscle protein of mice and guinea pigs infected with *Nematospiroides dubius* and *T. colubriformis* respectively. Their results indicated that with both infections the synthesis of muscle protein was depressed whereas hepatic protein synthesis was increased. In infected guinea pigs changes in nucleic acid concentrations and the sedimentation pattern of ribosomes from both tissues were consistent with the results of the amino acid incorporation study. The loss of weight that occurred in infected mice closely parallelled inappetance, but was not solely responsible for the changes in tissue protein synthesis; incorporation of ^{14}C-leucine into muscle protein was similarly depressed in non-infected mice made to lose weight at a comparable rate by reducing their feed intake, but there was no increase in the incorporation of amino acid into liver protein (Symons and Jones 1971).

Whole body disappearance of radioactivity following injection of ^{75}Se-selenomethionine into mice has indicated that an overall increase in protein turnover results from infection with *N. dubius* (Symons and Jones 1972). The half-life of selenomethionine was 17.4d in infected mice which were losing weight, compared to 26.2d in worm-free animals gaining weight. Similar measurements in uninfected mice on reduced feed intakes suggested that the increased protein catabolism and associated loss of weight in infected mice largely stemmed from inappetance.

Attempts have been made recently to explain increases in the rate of liver protein synthesis in guinea pigs infected with *T. colubriformis* through investigations at both the cellular and whole-animal levels (Symons, Jones and Steel 1973).

Measurements in vitro of the incorporation of ^{14}C-leucine into polypeptides by hepatic ribosomes showed that increased rates of protein synthesis by preparations from infected animals were primarily associated with membrane-bound ribosomes rather than free ribosomes. Inappetance of infected animals was not responsible for this increase. Membrane-bound ribosomes are known to synthesize the circulating plasma proteins; plasma albumin catabolism and loss into the intestine were both found to be significantly increased in similarly infected guinea pigs. It was concluded that hepatic protein synthesis by membrane-bound ribosomes was stimulated by these changes in albumin metabolism.

Although direct evidence is not available, it seems likely that similar changes in tissue protein metabolism occur in ruminants as a consequence of the effects of gastrointestinal nematodes on plasma protein turnover and thereby lead to impaired meat and wool production. This view is substantiated by recent investigations of the endocrine response in sheep to infection with *T.colubriformis*. Prichard, Hennessy and Griffiths (1973) found that plasma corticosteroid levels rose to a greater degree in infected sheep than in pair-fed, worm-free controls. Insulin levels were depressed to a similar degree in both groups when feed intake was reduced The known effects of these hormones led the authors to conclude that these changes were consistent with an impairment of muscle protein synthesis and an increased liver protein synthesis. *Furthermore following infection, plasma thyroxine concentrations were reduced whereas pair-fed animals exhibited no change; these changes together with the elevated corticosteroid concentrations are consistent with the depression of wool growth known to occur in sheep with trichostrongylosis.

In conclusion, the available evidence strongly suggests that impaired productivity in ruminants infected with gastrointestinal nematodes is primarily a result of disturbances to the nitrogen economy of the host. The primary physiological lesion common to these infections appears to be the leakage of plasma proteins into the gut, presumably in response to the mucosal damage caused by the worms. Quantitative studies are required to determine the degree to which this leakage influences the availability of amino nitrogen at the site of protein synthesis, and thereby compounds the effects of inappetance in limiting the production of both meat and wool.

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