The effect of two levels of dietary protein on resistance and resilience of dairy goats experimentally infected with \textit{Trichostrongylus colubriformis}: comparison between high and low producers

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Abstract – Numerous studies have examined the interactions between protein nutrition and the response to nematode parasitism in sheep, but very few in goats. Compared with other ruminants, goats are less resistant to nematode infection. In addition, in dairy goats, high producing animals have been shown to be less resistant and less resilient to infection compared to low producing ones. The objective of the present study was to examine the consequences of protein supplementation on both resistance and resilience of dairy goats to nematode trickle infection, taking into account the initial level of milk production of the animals. During a 14-week period, 40 milking goats received a high protein (HP) diet supplying 130% of the protein requirements, and 38 goats were fed a intermediate protein (IP) diet (120% of the protein requirements). In addition, half of each group was given a weekly trickle infection with \textit{Trichostrongylus colubriformis} larvae, the other part of the flock remained non-infected. Faecal egg counts (FEC), eosinophil counts and pathophysiological data (urea, albumin and inorganic phosphate concentrations in the serum) were measured twice a month. Milk parameters (related to resilience) were not affected by the level of protein in the diet when considering the whole groups. In contrast, in the high producing goats, the milk production and milk composition parameters were improved with the HP diet. To conclude, we have seen that the expression...
of both resistance and resilience did not appear when the coverage of the protein requirements was insufficient. Because the milk production is dependent on the protein supply, we suggest that there is a competition in the use of the protein between the development of resistance and the milk production.

*Trichostrongylus colubriformis* / nematode / protein supplementation / goat / milk production

Résumé – Effet de deux niveaux d’alimentation protéique sur la résistance et la résilience de chèvres laitières infestées expérimentalement avec *Trichostrongylus colubriformis* : comparaison entre fortes et faibles productrices. Alors que les études concernant les interactions entre l’alimentation protéique et la réponse au parasitisme gastro-intestinal sont nombreuses chez le mouton, elles le sont beaucoup moins chez la chèvre. Comparativement aux autres ruminants, les chèvres sont moins résistantes aux nématodes parasites. De plus, chez la chèvre laitière, il a été montré que les animaux forts producteurs de lait avaient une moins bonne résistance et une moins bonne résilience que les animaux faibles producteurs. Le but du travail ici présenté était l’étude des conséquences d’une supplémentation protéique sur la résistance et la résilience de chèvres laitières soumises à une infestation répétée à nématodes, étude qui tient compte du niveau de production initial des animaux. 

Durant 14 semaines 40 chèvres en lactation ont reçu une ration à haut niveau protéique couvrant 130% des besoins protéiques, et 38 chèvres furent alimentées avec une ration à niveau protéique moyen (120% des besoins protéiques). De plus, la moitié de chaque groupe fut infestée hebdomadairement avec des larves *Trichostrongylus colubriformis* (10000 L3), les autres animaux restant non parasités. Un comptage des œufs, des éosinophiles sanguins et une mesure de données physiologiques (concentration d’urée, d’albumine et d’phosphate dans le sérum) furent réalisés deux fois par mois. Les données de production (lait, protéines et matières grasses dans le lait) furent enregistrées, également tous les 15 jours. Le nombre d’œufs de parasite était significativement (p < 0.05) plus bas et le nombre d’éosinophiles sanguins était significativement (p < 0.05) plus élevé chez les animaux recevant la ration à haut niveau protéique, ce qui montre que la supplémentation protéique permet une amélioration de la résistance. Si l’on considère les données relatives au lait (reflet de la résilience) sur l’ensemble de l’échantillon, elles n’ont pas été modifiées par le niveau protéique de la ration. En revanche, chez les chèvres fortes productrices, l’augmentation du niveau protéique de la ration a amélioré les paramètres de production laitière et de composition du lait des animaux parasités. Ces résultats montrent que l’expression de la résistance et de la résilience ne peut apparaître que lorsque les besoins protéiques ne sont pas couverts. Le niveau de production laitière étant dépendant de l’apport protéique, nous émettons l’hypothèse d’une compétition dans l’utilisation des protéines entre le développement de la résistance d’une part et la production de lait d’autre part.

*Trichostrongylus colubriformis* / nematode / supplémentation protéique / chèvre / production laitière

1. INTRODUCTION

Several studies in sheep show that both resistance (i.e. the ability to limit worm burden) [1, 10] and resilience (i.e. the ability to maintain production despite parasitism) [24, 25] to nematode infection are influenced by diet, particularly by the metabolisable protein supply. Indeed Bown et al. [4] showed that gastrointestinal nematode infections induce protein losses rather than energy deficiency. However, there are very few studies in this field on goats and their results are equivocal: in growing goats, Blackburn et al. [2, 3] recorded an improvement in resilience with protein supplementation but no effect on resistance whereas Singh et al. [23] reported an enhancement of both resistance and resilience using a diet with cottonseed meal supplementation (reduction of faecal egg count and worm burden and no detrimental effect of parasitism on live-weight gain in young goats). It is worth underlining that these studies were performed with young, growing goats and that in fact, no studies have examined the influence of protein supplementation in dairy goats.

In *Trichostrongylus colubriformis* and *Haemonchus contortus* infected dairy goats,
a negative relationship was found between the level of milk production and individual responsiveness to gastrointestinal nematodes [8, 14]. There is a lack of resistance to parasitism in high producing goats whereas low producers develop a response to limit worm infection. Moreover, the consequences of infection in terms of milk production are more dramatic in high producers. If these differences are related to higher nutritional requirements in high producers, it could be hypothesised that differences in the diet protein supply should more dramatically affect the goats with the highest level of milk production compared to low producers.

The aim of this study was thus to investigate the influence of two levels of protein in the diet on the resistance and resilience of dairy goats to *T. colubriformis* infection, and particularly on the production level. The possible differences in the response of goats according to the initial level of milk production were also investigated.

## 2. MATERIALS AND METHODS

### 2.1 Experimental design (Fig. 1)

Seventy-eight Alpine dairy goats, reared indoors and previously free of strongyle infection were used in the study. Their lactation number was ≥2. At the start of the study, all the goats were in their second month of lactation.

Forty of these goats were offered a high protein diet (HP diet) during the experiment whereas the remaining thirty-eight received an intermediate protein diet (IP diet) during the same period. Half of each group was infected weekly with a dose of 10,000 *T. colubriformis* larvae, the others remained non-infected (control animals). The goats were ranked on the basis of milk production during the first month of lactation (a control was assessed at the end of the 1st lactation month) and they were allocated to 4 different balanced subgroups (HP diet...
infected and non infected; IP diet infected or not). The allocation into a parasite infected group was made by randomisation in order to have equilibrated groups according to weight, age and level of production.

In each diet-group, 10 high producers (5 infected and 5 control animals) and 10 low producers (5 infected and 5 control animals) were compared. The repartition in high or low producers was determined by the level of production at the first milk yield control. In the HP and IP diet group, the average production before infection amounted respectively to 4.85 (± 0.52) L day⁻¹ and 4.68 (± 0.6) L day⁻¹ in the high producers versus 2.76 (± 0.64) L day⁻¹ and 2.49 (± 0.66) L day⁻¹ for low producers.

2.2 Feeding and management

The ration offered to the animals was made up of two commercial concentrates, one cereal (corn), and forages (wheat straw, hay and fibrous pellets) (Tab. I). The variation in the proportion of the different components enabled the creation of two distinct rations. Samples of forages were weighed on 5 occasions during the study, on weeks 0, 3, 5, 6 and 9, in order to establish the real intake.

The HP and IP diets had very close concentrate:forage ratios (0.74 and 0.82). In each group, at the start of the experiment, the diets were isoeenergetic, containing 106% of the estimated average energy requirement [16]. The HP diet contained 130% of the estimated average protein requirements [16] and the IP diet 120% of the protein requirements. The 10% difference in covering the protein requirement was chosen since it represents the actual range in intensive dairy goat farms in France.

However, as the protein requirements are dependent on milk production, the difference in coverage was more dramatic in high producers vs. low producers. For example, at the start of the study, the IP diet provided 90% of the protein requirements for the high producers whereas the HP diet provided 147% for the low producers. In addition milk production varies with the stage of lactation. Hence, the evolution of requirement coverages according to the milk production are illustrated in Figure 2.

Since protein requirements are related to the stage of lactation and also to the level of milk production, the IP diet provided 90%, of the protein requirements for the high producers at the start and 115% at the end; that is an insufficient protein supply at the beginning of the experiment. In contrast, the HP diet provided between 110 and 145% of the protein requirements of high producing animals. For low producers the ration always broadly covered the needs whatever the diet (Fig. 2).

2.3 Parasitological techniques

All the animals were sampled prior to the start of the study and a faecal sample was thereafter collected from each animal

<table>
<thead>
<tr>
<th>Ration components</th>
<th>IP diet</th>
<th>HP diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial concentrate1 (kg d.m.)</td>
<td>0.420</td>
<td>0.538</td>
</tr>
<tr>
<td>Commercial concentrate2 (kg d.m.)</td>
<td>0</td>
<td>0.064</td>
</tr>
<tr>
<td>Corn (kg d.m.)</td>
<td>0.624</td>
<td>0.471</td>
</tr>
<tr>
<td>Wheat straw (kg d.m.)</td>
<td>0.081</td>
<td>0.084</td>
</tr>
<tr>
<td>Hay (kg d.m.)</td>
<td>0.534</td>
<td>0.513</td>
</tr>
<tr>
<td>Fibrous pellets (kg d.m.)</td>
<td>0.797</td>
<td>0.708</td>
</tr>
</tbody>
</table>
Protein supplementation and nematode infection in dairy goats

Every fortnight, faecal nematode egg counts were performed according to a modified MacMaster technique [21].

### 2.4 Blood analyses

Every two weeks, at the same hour of the day, jugular blood for haematological and biochemical analyses was collected in individual evacuated glass tubes: one heparinised tube was used to collect blood for the eosinophil count and one dry tube to collect blood in order to separate serum. Eosinophil numbers were determined using fast-read 102 slides and Carpentier’s solution according to the method described by Dawkins et al. [12]. Serum inorganic phosphate concentrations were determined using an autoanalyser (Technicon, Bayer Diagnostics Mfg SA, Margency, France) according to Robinson et al. [22]. Urea and albumin concentrations were also determined using the same autoanalyser.

### 2.5 Milk production data

Milk yield and fat and protein content data were individually collected twice a month at the morning milking by the local milk registration organisation. In the comparisons between groups, we used milk fat production and milk protein production (milk production by fat or protein content).

### 2.6 Statistical analysis

For faecal egg count values, a non-parametric test (Mann and Whitney test) was used to compare, date by date, the effects of the diet and of the initial level of milk production on the infection.

For eosinophils and production parameters, a global 2-way analysis of variance (ANOVA 2) was applied to analyse the effect of parasitism and diet, and the possible interaction. When there was a significant difference for the first measurement, analysis of covariance (ANCOVA) was assessed using the first measurement as the covariable. For eosinophils, data were transformed \[\log (\text{number of eosinophils} + 1)\] in order to stabilise the variance before running parametric tests.

The Student t-test was used date by date to compare the infected and control animals for each diet groups. In addition, within each diet group, the data values were also compared depending on the level of production.
Comparisons were done for eosinophil number, inorganic phosphate, urea, milk, total fat and protein production.

3. RESULTS

3.1 Faecal egg count (Fig. 3)

One month after the beginning of the trickle infection, the level of egg excretion was approximately 200–250 epg (eggs per gram) in each group. Then it showed a slight regular decrease until the end of the experiment for the goats receiving the HP diet. The pattern of faecal egg output of goats receiving the IP diet was similar to the one of those offered the HP diet during the first two months. For the third and last month, the goats with the HP ration excreted significantly (p < 0.05) less eggs than those with the IP ration.

3.2 Haematological and serological data

With the eosinophil count, the global ANOVA was significant for both the diet (first and sixth week) and parasitism (first week). In the IP diet group the eosinophil count showed an increase at the start of the study. Then the level decreased until the sixth week, when it showed a second peak before dropping. No difference between infected and non-infected goats was shown.

In HP diet group, eosinophil numbers of infected animals showed significantly (p < 0.05) higher values than the uninfected ones at the beginning of the experiment. Therefore an ANCOVA was also performed using the data of the first sample as covariable. A significant statistical difference between infected and non-infected goats on the twelfth week (p < 0.05) was also shown in the HP diet group (Fig. 4).

In high producing goats, the difference between infected and non-infected animals at week 12 was found whatever the diet. In low-producers no difference was found between infected and non-infected animals for the IP diet as for the HP diet (data not shown).

The inorganic phosphate concentration in the serum showed a similar evolution whatever the diet. The curve showed a regular increase until week 12 and then decreased until the end of the experiment.
The only significant difference concerned the HP group and appeared between infected and non-infected goats during the twelfth week of infection. The infected animals showed a lower inorganic phosphate concentration than the non-infected ones.

Measurement of urea showed no difference according to the parasite infection and the diet. However, after the first month of infection the HP group showed non-significant higher urea levels than the IP group.

3.3 Milk yield and composition data

For milk yield the global ANOVA 2 was not significant for diet nor for parasitism (Fig. 5).

At the beginning of the experiment, the average milk production for the whole flock was 3.7 (± 0.9) L-day⁻¹ and, at the end, it was 2.9 (± 0.9) L-day⁻¹. The milk yield showed an expected regular decrease over the three months.
However, the milk production curves were clearly different in the high and the low producers (Fig. 6). In the low-producers, production remained more or less the same throughout the whole experiment, while the high-producers had an important decrease. Nevertheless this production stayed higher than that of the low-producers (3.25 vs. 2.25 at the end of the experiment).

For the high-producers receiving the IP diet, significant differences ($p < 0.01$) in milk production due to nematode infection were observed at weeks 4 and 6. Concerning the high-producers receiving the HP diet, no significant difference in milk yield was recorded between infected and non-infected animals.

For milk fat production, the global ANOVA 2 was not significant for parasitism, but differences appeared with protein levels. The milk fat production in the high-protein diet group remained at a higher level than for the low-protein diet group (145 g day$^{-1}$ vs. 125 g day$^{-1}$) during the second month.

**Figure 6.** Milk yield of goats with the intermediate protein diet (IP) and the high protein diet (HP) in high producers and low producers.
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The global ANOVA 2 showed no interaction between parasitism and diet. The milk fat production curve was similar to the milk yield one and declined from 160 (± 44) g·day⁻¹ to 108 (± 36) g·day⁻¹ at the end of the experiment.

Milk fat production curves for high- and low-producers had the same profile than the milk yield ones. With high-producers (Fig. 7), the significant difference (p < 0.05) appeared at week 4 between infected and non-infected IP diet goats. In intermediate protein diet group, there was no influence of parasitism on fat production for the low producers.

A pattern similar to that of fat production, was seen with regards to milk protein production which declined from 127 (± 30) g·day⁻¹ to 97 (± 27) g·day⁻¹. Neither ration nor parasitism had any influence on this parameter and there was no interaction between the two factors.

In the milk protein production curves, significant differences were found at week 4 (p < 0.001) and week 6 (p < 0.05) measurements for the high-producers receiving the IP diet (Fig. 8). There was no drop in milk protein production for the low producers due to the parasitism.

4. DISCUSSION

There are many studies on the influence of diet, and particularly on protein level, on the resistance and the resilience of domestic ruminants to gastrointestinal nematode infection (see review by Poppi et al., [20] and [9, 25]). However, most of the studies concern growing animals, principally sheep, bred to produce meat or wool fiber. Only few studies have examined the role of diet on the response to parasites in milking animals despite the clear relationship between lactation and nutrition [13]. To our knowledge, the present results represent the first report on the interaction between protein diet and the response to parasite infection in dairy goats.

Significant differences in the level of faecal excretion were found between the 2 diet groups after 2 months of infection. The goats fed the HP diet excreted less eggs than the animals receiving the IP ration. This result suggests a better expression of resistance of the animals with the HP regime. This idea seems to be confirmed by the blood eosinophil data. Significant increases in the number of blood eosinophil cells were observed only in the HP group. According to
Dawkins et al. [12], Buddle et al. [5] and Kyriazakis et al. [17] for *T. colubriformis* infected sheep or Patterson et al. [18, 19] for infected goats, a rise in blood eosinophils is associated with the immune responsiveness of the animals to parasites. Hence, in the current study, the HP diet appeared to allow a better expression of the immune response in the infected goats.

In sheep, an increase in the level of digestible protein of the diet has been frequently associated with an improvement of the resistance to nematodes, assessed through different measurements: that is egg excretion, worm counts or blood eosinophil number. Such results have been recorded in lambs infected with the main species of the trichostrongyle nematode: *Teladorsagia circumcincta* [10]; *T. colubriformis* [24] or *H. contortus* [1, 11]. In contrast, there are few studies on goats and they provide equivocal results. Blackburn et al. [2, 3] did not observe any effect of protein supplementation on the worm burdens in young meat producing goats infected with *H. contortus*. In contrast, in growing goats parasitised with *T. colubriformis*, Singh et al. [23] showed a reduction in FEC and worm burdens in animals receiving a supplemented diet. This divergence in the results could be related to the worm species. Indeed, in mixed infected adult goats, Charles et al. [6] showed that different diets (difference of 68 g of cottonseed cake between the HP and IP diet) influenced the response to *Oesophagostomum columbianum* but do not modify the establishment of the other nematode species, *T. colubriformis*, *T. axei* and *H. contortus*.

In sheep, the better immune response in parasitised animals associated with protein supplementation leads to a decreased worm fecundity and/or to a reduction in worm populations in the digestive tract. This last observation can result from a less efficient rate of parasite establishment and/or from an increased expulsion of the worms. Coop and Holmes [9] and Van Houtert et Sykes [25] reported that in young sheep, nematode establishment is not appreciably affected by diet. On the contrary, the rate of parasite elimination is enhanced in animals fed an improved diet, which explains most of the reduction in worm burden. In our study, no data (worm count) were available to directly measure the influence of diet on the worm population. However, an indirect estimation was provided by serum

![Figure 8. Milk protein production of goats with the intermediate protein diet (IP) and the high protein diet (HP) in high producers.](image)
concentrations of inorganic phosphate whose changes are related to the pathophysiological consequences of infection due to the presence of intestinal worm populations. No difference in the inorganic phosphate values was detected during the study between the infected goats under the different diets which suggest only a minor difference in the size of worm populations. Therefore, the observed differences in egg excretion probably result mainly from a reduction in worm fecundity.

An increase in the level of digestible protein in the diet has usually been associated both with an improvement of resistance and a better resilience of the animals. In the present study, when considering the whole flock, parasitism did not provoke any effect on the recorded milk production parameters and no difference was detected between the 2 protein diet groups. This could be explained by the probable low level of parasite establishment. It is, however, worthy to note that the consequences on production due to infection diverged when one discriminated the high- and the low-producers within the flock.

In the high producers, the presence of *T. colubriformis* induced constant and significant decreases in milk yield, milk fat and milk protein yields, particularly at the start of the experiment. In contrast, in the low producers, whatever the diet regime, parasite infection did not have any consequence on the 3 milk production parameters. This higher susceptibility of the high-producing goats to nematode infection has previously been assessed in experimental [8, 14, 15] or natural infection [7]. Two hypotheses have been proposed to explain the lesser resistance and resilience of high producing dairy goats to nematodes: (i) either a genetic origin or (ii) a consequence of the nutritional/physiological stress due to lactation. Considering this last hypothesis, the comparison of the response to *T. colubriformis* infection in the high producers with the 2 different diets is particularly interesting. The effects of infection on milk production parameters were clearly more pronounced in the group of high producers fed the IP diet. In addition, the significant differences between infected and control goats were usually found at the start of the experiment when the coverage of protein requirements were insufficient (see Fig. 2). These results suggest that the origin of the increased susceptibility to parasitism in the high producing animals can partly be related to a possible competition between the production of milk and the development of resistance. In their recent review, Coop and Holmes [9] propose a hypothesis concerning competition between the requirements to mount an immune response to parasites and the needs for growth. These authors underlined the fact that improvements of the host response against parasites associated with changing the diet is more obvious in young lambs which have larger protein requirements.

Besides the possible application in terms of nutrition manipulation in improving the response of goats to parasites, our study also underlines the interest of examining the consequences of parasitoses for milking animals. The variability in milk production within a flock combined with the variation in nutritional requirements with time due to the evolution of the lactation curve can provide a useful model to understand the complex relationship between nutritional status of the host and its response to nematode infection.

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