Impact of the post-weaning nutritional history on the response to an experimental *Haemonchus contortus* infection in Creole goats and Black Belly sheep

W. Ceï¹, N. Salah¹, C. Paut¹, P.-J. Dumoulin², R. Arquet², Y. Félicité¹, G. Alexandre¹, H. Archimède¹, J.-C. Bambou¹,∗

¹ Institut National de la Recherche Agronomique, Unité de Recherches Zootéchniques, Domaine Duclos, 97170 Petit-Bourg (French West Indies), France
² Institut National de la Recherche Agronomique, Domaine expérimental de Cardel, 97160 Moule, Guadeloupe, France

**Abstract**

In small ruminants, the response against gastrointestinal nematode (GIN) infections is influenced not only by the host genotype and the physiological stage but also by environmental factors, particularly the nutritional status at the time of infection. In this study we evaluated the long-term effect and the interaction between the host species and the nutritional history on the response to GIN infection in two animal models differing in their phenotypic growth and their level of GIN resistance: Black Belly sheep and Creole goats. Lambs and kids were subjected to three distinct nutritional conditions at weaning: low dietary conditions (100% of the theoretical energy requirement for maintenance, corresponding to 548 v. 484 KJ/Kg BW⁰.⁷⁵ for lambs and kids respectively and 6% of crude protein, CP), medium dietary conditions (150% of the theoretical energy requirement for maintenance and 13% CP) and high dietary conditions (200% of the theoretical energy requirement for maintenance and 20% CP). This 3-months period was followed by a 1-month period on the medium dietary conditions for all the animals before an experimental *Haemonchus contortus* infection. We monitored the impact of the nutritional history (nutritional condition after weaning), on the intensity of the GIN infection by measuring individual faecal egg counts (FEC), growth rate (ADG), blood eosinophil counts and other pathophysiological parameters. The FEC, growth rate and blood eosinophil counts were significantly affected by the nutritional history in lambs but not in kids. The lowest FEC was found for lambs placed in high dietary conditions, however during the same period body weight loss was observed in that group. In low dietary conditions, kids were more resistant than lambs and the ADG was higher in lambs. However, the anaemia and the level of serum pepsinogen, marker of the abomasal mucosa integrity, were higher in kids. Our data suggest that the impact of the post-weaning nutritional history on the response to an experimental *H. contortus* infection is significantly affected by the host species.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The constant increase of the prevalence of anthelmintic-resistant gastrointestinal nematode (GIN) strains together with an increasing demand of chemical-free animal products and the potential environmental consequences of anthelmintics increase the importance of alternative control strategies (Beynon, 2012). The objective is no more the search for a unique solution to eradicate the GIN populations, but rather an integration of different control methods to reach a favorable equilibrium for animal production. Among these alternative strategies, the optimisation of the host nutrition to improve resistance and/or resilience to GIN infections is a short-term solution easy to implement (Torres-Acosta et al., 2012).

There is accumulating evidence showing that the nutritional status is closely associated with the capacity of the host to mount an efficient immune response against invading pathogens (Calder et al., 2002; Gershwin et al., 2004). Indeed, mounting an immune response is expensive both in terms of protein and energy because of the metabolic requirement of immune cells, the synthesis of proteinaceous immune mediators and the repairing of damaged tissues (Lord et al., 2001; Sykes, 2010). Thus, numerous studies...
have shown that the response to an immunological challenge must be traded off against other physiological functions such as reproduction, growth and thermoregulation (Sheldon and Verhulst, 1996; Shudo and Iwasa, 2001; van der Most et al., 2011; Zuk and Stoehr, 2002). Moreover, a singular feature of pathogens infection across mammalian and avian hosts is the reduction in voluntary food intake, at a time of increased nutritional demand (Kyriazakis et al., 1998). Recently, Kyriazakis put forward the hypothesis that, in herbivore anorexia can be viewed as both an unavoidable consequence of infection but also as a behavior strategy that enables them to cope with the consequences of infection (Kyriazakis, 2014). Nevertheless, benefits linked to pathogen-induced anorexia would probably require fine homeostatic control, as chronic undernutrition has deleterious consequences for host defense.

The trade-off between the major physiological functions, including the immune response against invading pathogens, is influenced not only by the host genotype and the physiological stage but also by environmental factors, particularly the availability and the quality of the feed in the ecosystem (Lochmiller and Deerenberg, 2000). By the use of a mathematical model, Vagenas et al. (2007a, b) showed a higher significant effect of the nutritional status on GIN resistance traits in sheep than the effect of the host genotype, suggesting that discrepancies between published genetic parameters for GIN resistance may be function of environmental factors rather than differences in host genotype. In sheep it has been shown that the nutritional status in early life significantly the resistance to GIN in the later life (Datta et al., 1999). However, data on the relationships between the nutritional status in early life and the resistance to GIN are still scarce in sheep and even more in goats. Hence, in this study we evaluated whether the nutritional history in early life would affect the resistance to an experimental GIN infection in two host species (i.e., sheep and goats).

2. Materials and methods

The experiment was conducted at the Institut National de la Recherche Agronomique (INRA) Animal Production Unit, Guadeloupe (French West Indies) (16° 16’ latitude North, 61° 30’ longitude West). All animal care, handling techniques, procedures as well as license for experimental infection and blood sampling were approved by INRA according to the certificate number A-971-18-02 of authorization to experiment on living animals issued by the French Ministry of Agriculture, before the initiation of the experiment.

2.1. Animals, management and experimental design

The study was carried out with two successive groups of 48 animals (n = 24 Black belly lambs; 25.3 ± 2.87 kg BW; 3 months old and n = 24 Creole kids; 12.6 ± 2.17 kg BW; 3 months old) during two trials. From birth to weaning the animals were raised in a rotational grazing system in which the pasture was rested for 4 weeks between two grazing periods of 5 days. At 3 months of age all the animals were weaned and drenched with ivermectin (Oramec, Merial, Lyon, France, 0.3 mg/kg BW) and toltrazuril (Baycox Ovis, Bayer HealthCare, Loos, France, 20 mg/kg BW). Each trial started at weaning. The first period lasted 105 days during which animals were randomly allocated to one of 3 distinct diets: Low, Medium and High dietary conditions. On the basis of the meta-analysis of Salah et al., 2014 the 3 dietary conditions were balanced in energy and protein as follow: (i) 100% of the theoretical energy requirement for maintenance (548 v. 484 KJ/kg BW0.75 for lambs and kids respectively) and 6% of crude protein (CP) (Low dietary condition), (ii) 150% energy requirement for maintenance (822 v. 726 KJ/kg BW0.75 for lambs and kids respectively) and 13% CP (Medium dietary condition) and, (iii) 200% energy requirement for maintenance (1096 v. 968 KJ/kg BW0.75 for lambs and kids respectively) and 20% CP (High dietary condition). The banana flour, the soya and the additives of the ration were distributed to the animals in the form of pellets (Table 1). During this period, all the animals were reared in individual pens (2 × 2 m) with free-choice access to fresh water and were weighed every 21 days to adjust the offered quantities according to body weight change (Table 1). At the end of this period, the animals were placed in 6 collective pens (for lambs and kids allocated to the 3 distinct diets respectively), and received the pellets corresponding to the medium dietary condition and hay ad libitum. After a 30 days period of adjustment to the collective pens and the diet conditions, all the animals were experimentally infected with a single oral dose of 10,000 H. contortus third-stage infective larvae (L3). The L3 were obtained 42 days before challenge from cultures of faeces taken from anthelmintic-susceptible strain harvested from faeces of monospecifically infected donor Creole goats with isolates previously obtained from Creole goats reared on pasture in different farms in Guadeloupe (Bambou et al., 2008). During the experimental infection the animals remained on the same diet corresponding to the medium dietary condition, and the measurement of BW changes, FEC and blood parameters were performed weekly during 5 consecutive weeks.

2.2. Feed intake and growth measurements

During the first period when animals were in individual pens, the average voluntary dry matter intake (DMI) was calculated individually as the difference between the daily amounts of feed offered and feed refusal. The BCS were measured weekly during the last 5 weeks of the first period by palpation of the lumbar and the sternal vertebrae and associated soft tissue. A scale of one (thin) to five (fat) was used and the BCS was the mean score of the lumbar and the sternal region. When animals were placed in collective pens the pellets were distributed first and individually with the help of yoke traps during the consumption lapses’ time. Thereafter, the hay was distributed ad libitum. Feeding stalls were long enough to avoid competition for hay between the kids. The offered hay was adjusted to the groups BW. During the infection period the average volun-

<table>
<thead>
<tr>
<th>Table 1 Composition and nutritional values of diets.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional conditions</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Ingredients (g/Kg DM²)</td>
</tr>
<tr>
<td>Hay</td>
</tr>
<tr>
<td>Banana flour</td>
</tr>
<tr>
<td>Soya</td>
</tr>
<tr>
<td>Premix</td>
</tr>
<tr>
<td>Bicalciyum</td>
</tr>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Chemical composition (g/Kg DM)</td>
</tr>
<tr>
<td>OM⁴</td>
</tr>
<tr>
<td>CP</td>
</tr>
<tr>
<td>NDF⁴</td>
</tr>
<tr>
<td>ADP⁴</td>
</tr>
<tr>
<td>ADL⁴</td>
</tr>
<tr>
<td>ME⁴ (MJ/Kg DM)</td>
</tr>
<tr>
<td>DMI⁵ (Kg BW⁵)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Description</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>Dry matter</td>
<td>500</td>
<td>582</td>
<td>250</td>
</tr>
<tr>
<td>Banana flour</td>
<td>Organic matter</td>
<td>482</td>
<td>250</td>
<td>368</td>
</tr>
<tr>
<td>Soya</td>
<td>Crude protein</td>
<td>0</td>
<td>150</td>
<td>364</td>
</tr>
<tr>
<td>Premix</td>
<td>Neutral detergent fiber</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Bicalciyum</td>
<td>Acid detergent fiber</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>Acid detergent lignin</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>ME</td>
<td>Metabolisable energy</td>
<td>8.7</td>
<td>8.5</td>
<td>11.8</td>
</tr>
<tr>
<td>DMI</td>
<td>Body weight</td>
<td>26.0</td>
<td>40.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>
tary DMI calculated by group as the difference between the offered and the refused feed were significantly different.

2.3. Faecal eggs count

To determine FEC, faecal samples of approximately 10 g were weekly collected during experimental infection directly from the rectum of each animal. The faeces were kept in plastic tubes to avoid contamination and immediately transported to the laboratory in refrigerated vials. All samples were individually analyzed using a modified McMaster method for rapid determination and FEC was expressed as the number of eggs/g faeces.

2.4. Blood analysis

During the experimental infection, blood samples were individually collected once a week by jugular venipuncture from each animal by using disposable syringes and 20-Ga needles. A 2.5-ml portion of each blood sample was placed in commercial anticoagulant tubes (ethylenediamine tetraacetic acid K3, EDTA tubes; Becton Dickinson, Plymouth, UK). Blood samples previously placed in EDTA coated tubes were used to measure the number of circulating eosinophils according to the method of Dawkins et al. (Dawkins et al., 1989) with a Malassez cell counter. The packed cell volume (PCV) was measured using the capillary microhaematocrit method. Blood samples collected in plastic serum tubes (serum tubes; Becton Dickinson) were centrifuged for 5 min. at 5000 rpm then serum were frozen at −20 °C until analysis. Serum pepsinogen levels were determined using a micromethod for routine determination according to Dorny and Verbruggen (1998). The level of serum pepsinogen considered as an indicator of the mucosal damage caused by H. contortus infection was measured weekly. The measurement of total serum protein was performed by the Bradford method (Bradford, 1976). For serum pepsinogen and total serum proteins, each sample was evaluated in triplicate for each time point, a coefficient of variation of ≤15% was considered acceptable.

2.5. Calculation and statistical analysis

All the animal variables were analysed by a linear mixed model of variance using the PROC MIXED of SAS (Version 9, SAS Inst., Inc., Cary, NC, 1999). Because of skewed distributions, FEC and eosinophilia variables were logarithm transformed (Ln (FEC + 15), Ln (Blood eosinophils + 1) respectively) to normalize residual variances. The model included fixed effects of the days post-infection (T), the dietary condition (D), the host species (H) and the significant interaction between the dietary condition (D) and the host species (H) as defined below:

\[ y_{ijkl} = \mu + D_i + a_{ij} + H_j + T_k + (DH)_{ij} + e_{ijkl} \]

where y is the observed values; \( \mu \) the overall mean; \( D_i \) the fixed effect of the dietary condition; \( H_j \) the fixed effect of the host species, \( T_k \) the fixed effect of the days post-infection, \( (DH)_{ij} \) the interaction of the dietary condition and the host species, \( a_{ij} \) is the random effect associated with the ith animal in dietary condition i and host species j and \( e_{ijkl} \) the random error. All the interactions were initially tested for all variables and only the \( (DH)_{ij} \) was statistically significant and retained in the model. An unstructured variance–covariance structure was used to model the covariance between two observations on the same animal. The same model was applied for all the animal variables. Pearson correlation coefficient (PROC CORR of SAS) was used to determine phenotypic correlations among variables. The growth (Average Daily Gain, ADG) of the animals were estimated by adjusting the weight curve with a linear model. Significance was declared at ≤5% of probability.

3. Results

3.1. Average daily gain and body condition score

The composition and nutritional values of the diets is shown in Table 1. During the period before the experimental infection the higher ADG and BCS were observed in the animals placed in the medium and the high dietary conditions and no difference was observed between these dietary conditions (Table 2). A significant interaction between species and dietary conditions was observed on the ADG during the experimental infection with H. contortus (Fig. 1, \( P = 0.001 \)). The ADG in kids were low in the 3 groups and were not significantly different between the 3 dietary conditions \( (P > 0.05, \text{Fig. 1}) \). In lambs, the ADG was significantly affected by the dietary conditions after the experimental infection (Fig. 1). Negative ADG were observed in lambs placed in the high and the medium dietary condition, whereas a compensatory growth was observed in lambs placed in the low dietary condition. No significant difference was observed between kids and lambs placed in the high dietary condition \( (P = 0.78) \). In contrast, the ADG were significantly higher in kids compared with lambs in the medium and low dietary condition \( (P < 0.01) \).

3.2. Blood and parasitological measures

Effect of the dietary conditions comparing kids and lambs on FEC is shown in Fig. 2. A significant interaction between species and dietary conditions was observed \( (P = 0.002) \). In kids, no difference for FEC was observed between the 3 dietary conditions \( (P > 0.05) \). In lambs, the FEC were significantly different in the 3 dietary conditions \( (P < 0.05) \). Lambs placed in the high dietary condition showed the lowest FEC and sheep in the low dietary condition showed the highest FEC. No difference was observed between kids and lambs placed in the medium dietary condition for FEC \( (P > 0.05) \). In the high dietary condition, FEC was significantly higher in kids compared with lambs \( (P = 0.002) \). In contrast in the low dietary condition it was the reverse, FEC was lower in kids compared with lambs \( (P = 0.04) \).

The Fig. 3 shows the LS means of blood eosinophil counts during the experimental infection according to the dietary conditions and the host species. The interaction between dietary conditions and species was significant \( (P = 0.0001) \). No difference was observed between kids and lambs placed in the medium dietary condition \( (P > 0.05) \), but in the high and the medium dietary conditions, means of blood eosinophil counts were significantly higher in kids com-
Table 2
Least square means of Average Daily Gain (ADG) and Body Condition Score (BCS) of kids and lambs according to the dietary conditions before the experimental infection.

<table>
<thead>
<tr>
<th></th>
<th>Kids</th>
<th></th>
<th>Lambs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>ADG (g/day)</td>
<td>−9.55 (4.02)</td>
<td>63.72 (3.4)</td>
<td>69.62 (3.9)</td>
<td>14.12 (3.6)</td>
</tr>
<tr>
<td>BCS</td>
<td>1.25 (0.08)</td>
<td>2.49 (0.07)</td>
<td>2.39 (0.08)</td>
<td>1.29 (0.08)</td>
</tr>
</tbody>
</table>

* Means with different superscripts within each line differ significantly (P<0.05).

Fig. 2. Least square means and standard errors of log transformed faecal egg counts (FEC) of kids and lambs (Kids, lambs) according to the dietary condition (Low, Medium and High dietary conditions; n=8/dietary condition/species).

Fig. 3. Least square means and standard errors of log transformed blood eosinophil counts of kids and lambs (Kids, lambs) according to the dietary condition (Low, Medium and High dietary conditions; n=8/dietary condition/species).

Fig. 4. Least square means of packed cell volume (PCV) in kids and lambs according to the experimental groups: Low dietary condition (Kids, lambs), Medium dietary condition (Kids, lambs) and High dietary condition (Kids, lambs). The solid line represents the mean values for kids (-----) and the hatched line the mean values for lambs (--.--).

Fig. 5. Least square means of serum pepsinogen in kids and lambs according to the experimental groups: Low dietary condition (Kids, lambs), Medium dietary condition (Kids, lambs) and High dietary condition (Kids, lambs). The solid line represents the mean values for kids (-----) and the hatched line the mean values for lambs (--.--).

Fig. 6. Least square means of total serum protein concentrations in kids and lambs according to the experimental groups: Low dietary condition (Kids, lambs), Medium dietary condition (Kids, lambs) and High dietary condition (Kids, lambs). The solid line, the hatched line and the dotted line represent respectively the mean values for the Low dietary conditions groups (-----), the Medium dietary condition groups (-----) and the High dietary condition groups (-----).

The anaemia and the mucosal injuries caused by the blood feeding nature of H. contortus were monitored respectively through the measurement of the PCV and the serum pepsinogen during the course of the experimental infection (Figs. 4 and 5). No interaction between the dietary conditions and the species and no significant effect of the 3 dietary conditions were observed for the PCV and the serum pepsinogen level. However, a significant effect of the host species was observed (P<0.001). The PCV decreased in both species from 14 days post-infection to reach a mean value of 21% for kids and 26% for lambs from 21 post-infection (Fig. 4). The serum pepsinogen level was higher in kids compared with lambs (Fig. 5). While the serum pepsinogen increased transiently in sheep at 7 and 14 days post-infection, in kids it increased at 7 days post-infection and remained at this level.

During the course of the experimental infection, no significant interaction between the host species and the dietary conditions and no effect of the host species were observed for the serum protein concentration (Fig. 6). In contrast, the dietary conditions affect significantly the serum protein level (P<0.002). It decreased significantly whatever the host species at 7 and 14 days.
post-infection \((P < 0.01, \text{Fig. 6})\). Thereafter, the serum protein concentration increased to reach at 35 days post-infection the values observed in non-infected animals (day 0 post-infection). Kids and lambs placed in the low dietary condition showed the highest decreased in serum protein concentration during the course of the infection.

4. Discussion

Numerous feeding trials with small ruminants have paid much attention on the direct or indirect effects of an increased nutrients supply (in protein and/or energy) on the host response to GIN infections (Houdijk et al., 2012). However, few studies have addressed the issue of long-term effects of the nutritional history on the host response to GIN infections (Knox et al., 2003). In this study, we investigated the effect of the nutritional history during the early post-weaning period on the later response to an \(H. \ contortus\) infection by comparing two small ruminant species (i.e. goats and sheep). We showed an interaction between the host species and the nutritional history for growth and the response against the invading pathogen (i.e., \(H. \ contortus\)). The response against \(H. \ contortus\), monitored through the FEC, the blood eosinophil counts and the growth rate were significantly affected by the nutritional history in lambs but not in kids. The lowest FEC was found for lambs placed in high dietary condition. In this group body weight loss was observed in lambs but not in kids. Among animals placed in low dietary condition, kids were more resistant than lambs and the ADG was higher in lambs. Interestingly, a significant phenotypic negative correlation \((r = -0.49, \ P < 0.001)\), was found in lambs between FEC and blood eosinophil counts but not for kids. This result support the hypothesis of an increased immune response in lambs placed in high dietary condition. Indeed, here we considered that blood eosinophil counts could be a marker of the host response in accordance with previous studies which showed that eosinophils play a role in resistance to helminth infection since significant correlations between resistance/susceptibility to endoparasite infection and the magnitude of the peripheral eosinophil response have been shown (Meeusen et al., 2005). Furthermore, an increased blood eosinophilia after GIN infection have been observed in sheep and goats concomitantly supplemented (Valderrabano et al., 2002; Vanhoutert et al., 1995). In agreement with previous studies in Creole kids, the blood eosinophil counts were significantly higher in kids with the higher FEC but no phenotypic correlation was found (Bambou et al., 2008; Bambou et al., 2009). This result suggested that, despite the nutritional cost the blood eosinophil response could be considered as a marker of the level of infection in Creole kids rather than a marker of the protective response as observed in lambs. Furthermore, these results suggest a significant effect of the nutritional history on a potential trade-off for growth against the immune response in lambs but not in kids.

The hematophagous feeding of larval and adult stages of \(H. \ contortus\) on the abomasal mucosa leads to severe mucosal damages, anaemia, loss of serum proteins and oedema (Rahman and Collins, 1990; Rowe et al., 1988). In this study, the anaemia and the level of serum pepsinogen (marker of the abomasal mucosa integrity), were higher in kids. The serum pepsinogen increased more rapidly in kids, suggesting a delayed immune response against the incoming larvae which caused the mucosal injuries compared with lambs. No difference between kids and lambs was observed in the slight transient hypoproteinaemia induced after infection. However, the loss of serum protein was significantly affected by the host nutritional history, animals placed in medium and high dietary conditions showed a lower loss of serum proteins.

In lambs, similar trials showed that a short-term nutritional supplementation early after weaning potentiated a long-term resistance to GIN infection but in contrast with the present study no evidence for compensatory growth was observed for the animals placed in lower dietary condition (Datta et al., 1999). It has been shown for a long time that in growing animals a period of nutritional restriction generates a compensatory growth which depends on the nutritional conditions during the rehabilitation period (Kyriazakis and Emmans, 1992). This compensatory growth phenomenon is the result of the optimisation of different physiological functions of the animal whose main objective is to increase the growth rate (Hoch et al., 2003). In our trial, compensatory growth was observed in lambs showing the lowest response to \(H. \ contortus\) (higher FEC and lower eosinophilia). By using mathematical models, it has been shown that the host nutritional status could affect the interrelationship between host growth and resistance to pathogens (Doeschl-Wilson et al., 2009; Vagenas et al., 2007b). In contrast with our trial, in these models the infections were considered concomitantly with the host nutritional status. Interestingly, here we showed that the nutritional history could also impact the response of the host to a nematode infection and this effect is host-dependent. Indeed, in kids no effect of the nutritional history was observed on the response against the nematode infection and the growth rate.

In conclusion, our results suggest a potential trade-off between growth and the response against GIN significantly affected by the host species and the nutritional history. In low dietary condition, the priority of the Black Belly lambs was the growth function at the expense of the response against a GIN infection, whereas in the high dietary condition the priority was the response against the GIN infection. In contrast, despite a severe pathophysiological impact, the Creole kids used the robustness strategy; their priority was the growth during the GIN infection whatever the nutritional history.

Acknowledgments

The authors want to give thanks to C. Barbier, C. Deloumeaux and F. Labirin for care and handling of the animals. The authors are grateful to the Gardel team in charge of the goat flock: R. Arquet, T. Kandassamy, W. Troupé, J. Gobardhan and S.-A. Matou. This study was funded by La Région Guadeloupe and the INRA métaprogramme GISA (Gestion Intégrée de la Santé Animale) Project Strep (Drastic and Sustainable Treatment Reduction Against Parasitism in livestock). WC was supported by a doctoral fellowship from la Région Guadeloupe and the animal Genetics Department of INRA.

References


