# Impacts des interactions entre le statut nutritionnel et le parasitisme gastro-intestinal sur les réponses animales chez les petits ruminants

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# THÈSE DE DOCTORAT

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## Impacts des interactions entre le statut nutritionnel et le parasitisme gastro-intestinal sur les réponses animales chez les petits ruminants

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SGI : Strongles Gastro-Intestinaux

NGI : Nématode Gastro Intestinaux

INRA : Institut National de la Recherche Agronomique

L3 : larve stade 3 ou larve infestante

L5 : larve stade 5 ou adulte

*H.contortus* : *Haemonchus contortus*

OPG : œuf par gramme de fèces

Cf : se référer à

*T. circumcincta : Teladorsagia circumcincta*

$h^2$ : Héritabilité

CPA : Cellule Présentatrice d'Antigène

TCR : T-Cell Receptor

Th1: T Cell Helper 1

Th2: T Cell Helper 2

IL-: Interleukine –

Ig: Immunoglobuline

BW : Body Weight

PV : poids vif

i.e : c'est-à-dire

e.g : par exemple

AA : acides aminés

PAL : Phénylalanine Ammonia-Lyase

ASAT : Aspartate Amino Transférase

ALAT : Alanine Amino Transférase

↗ : augmente

↙ : diminue

# RÉSUMÉ

Les infestations par les strongles gastro-intestinaux (SGI) constituent une cause majeure de morbidité et de mortalité chez les petits ruminants élevés au pâturage et entraînent donc des pertes de production. En raison de l'émergence de la résistance aux anthelminthiques et des préoccupations des consommateurs concernant les résidus chimiques dans les produits animaux, des stratégies alternatives de contrôle sont nécessaires. L'objectif n'est plus l'éradication totale de la population parasitaire, mais plutôt un meilleur contrôle de ces populations pour atteindre un équilibre favorable à la production animale entre l'hôte et les parasites. Une stratégie prometteuse pour le contrôle des SGI est l'amélioration de la réponse de l'hôte par le statut nutritionnel des animaux. Bien que les chèvres soient plus sensibles que les moutons aux infestations par les NGI, la plupart des programmes de recherche visant à caractériser les interactions hôtes-NGI est menée chez les ovins. Contrairement à l'idée que les résultats acquis chez le mouton seraient applicables aux chèvres du fait de leur proximité, de nombreuses études soulignent des différences significatives entre ces modèles. L'objectif de ce travail de thèse était d'étudier l'impact du statut nutritionnel en termes de quantité de protéines et d'énergie et de la qualité des protéines apportée par la ration sur les réponses (production vs réponse contre le parasitisme) de caprins Créole infestés expérimentalement par *Haemonchus contortus*. Nous avons montré que le statut nutritionnel permettait de réduire la sévérité et la durée de l'anémie régénérative et de la thrombopénie induite par *H. contortus* chez le caprin Créole. La ration enrichie en protéines était associée à de la résilience vis-à-vis de l'infestation plutôt que de la résistance. Le mécanisme sous-jacent serait lié à une augmentation de la capacité régénérative de la moelle osseuse. Par contre, une augmentation de la part de protéines by-pass dans la ration était associée à de la résistance. Une augmentation de la réponse humorale IgA dirigée contre les larves L3 et les produits d'excrétions sécrétés par les parasites adultes était associée à cette résistance. Nous avons aussi cherché à valider l'hypothèse d'une partition différenciée des flux d'acides aminés avec le stress provoqué par *H. contortus*. Nous n'avons pas mis en évidence de différences de synthèses protéiques entre tissus et pour un même tissu entre animaux infestés et non infestés. En résumé, les travaux conduits dans cette thèse ont montré qu'il existe un équilibre entre la quantité et la qualité des protéines alimentaires de la ration qui permet d'améliorer la réponse des caprins Créole contre *H. contortus* ainsi que leurs performances de production.

# ABSTRACT

Gastrointestinal nematode (GIN) infections are a major cause of morbidity and mortality in small ruminants at pasture and therefore lead to production losses. Due to the emergence of anthelmintic resistance and consumer concerns about chemical residues in animal products, alternative control strategies are needed. The objective is no longer the total eradication of the parasite population, but rather a better control of these populations to achieve a favorable balance between the host and the parasites to improve animal production. A promising strategy for NGI control is to improve the host response through the nutritional status of the animals. Although goats are more susceptible than sheep to NGI infestations, most research programs to characterize host-NGI interactions are conducted in sheep. In contrast with the idea that the results obtained in sheep will be applicable to goats because of their proximity, many studies highlight significant differences between these models. The objective of this research project was to study the impact of nutritional status in terms of protein and energy quantity and protein quality provided by the diet on the responses (production vs response against parasitism) of Creole goats experimentally infested by *Haemonchus contortus*. We have shown that nutritional status reduces the severity and duration of regenerative anemia and *H. contortus*-induced thrombocytopenia in Creole goats. The protein-enriched diet was associated with resilience to infection rather than resistance. The underlying mechanism would be linked to an increase in the regenerative capacity of the bone marrow. However, an increase in by-pass protein of the diet was associated with resistance. An increase in the IgA humoral response directed against L3 larvae and secreted-excreted products of adult parasites was associated with this resistance. We also sought to validate the hypothesis of a differentiated partition of amino acid flows with the stress caused by *H. contortus*. We did not find differences in protein synthesis between tissues and for the same tissue between infected and non-infested animals. In summary, the work carried out in this PhD project show that there is a balance between the quantity and quality of dietary proteins in the diet which makes it possible to improve the response of Creole goats against *H. contortus* as well as their production performances.

# INTRODUCTION

## 1. Contexte du projet

Les pathologies liées aux strongles gastro-intestinaux (SGI) provoquent d'importantes pertes dans les élevages de petits ruminants sous toutes les latitudes. Cependant, en raison des conditions climatiques particulièrement favorables, la prévalence de SGI est élevée dans la région intertropicale. En Guadeloupe, la mortalité due aux infestations par les SGI des jeunes caprins avant le sevrage atteint 40% (Aumont 1997). D'après une étude réalisée en station INRA Antilles-Guyane, la mortalité des caprins Créole sans traitement anthelminthique est estimée à 16% entre le sevrage et 260 jours post-sevrage (Mandonnet et al., 2003). La lutte contre ces pathologies ne peut plus s'appuyer uniquement sur l'utilisation des anthelminthiques chimiques de synthèse du fait du développement rapide de la résistance des SGI aux principales molécules chimiques (Papadopoulos, 2008). Des stratégies de lutte intégrée (Figure 1.) associant l'utilisation ciblée de molécules chimiques, l'alimentation, la sélection génétique d'animaux résistants et/ou résilients, l'utilisation de plantes aux propriétés anthelminthiques, l'association d'animaux et la gestion alternative des pâturages se sont développées au cours de ces dernières années. Quelle que soit l'option retenue, la stratégie alimentaire demeure essentielle.

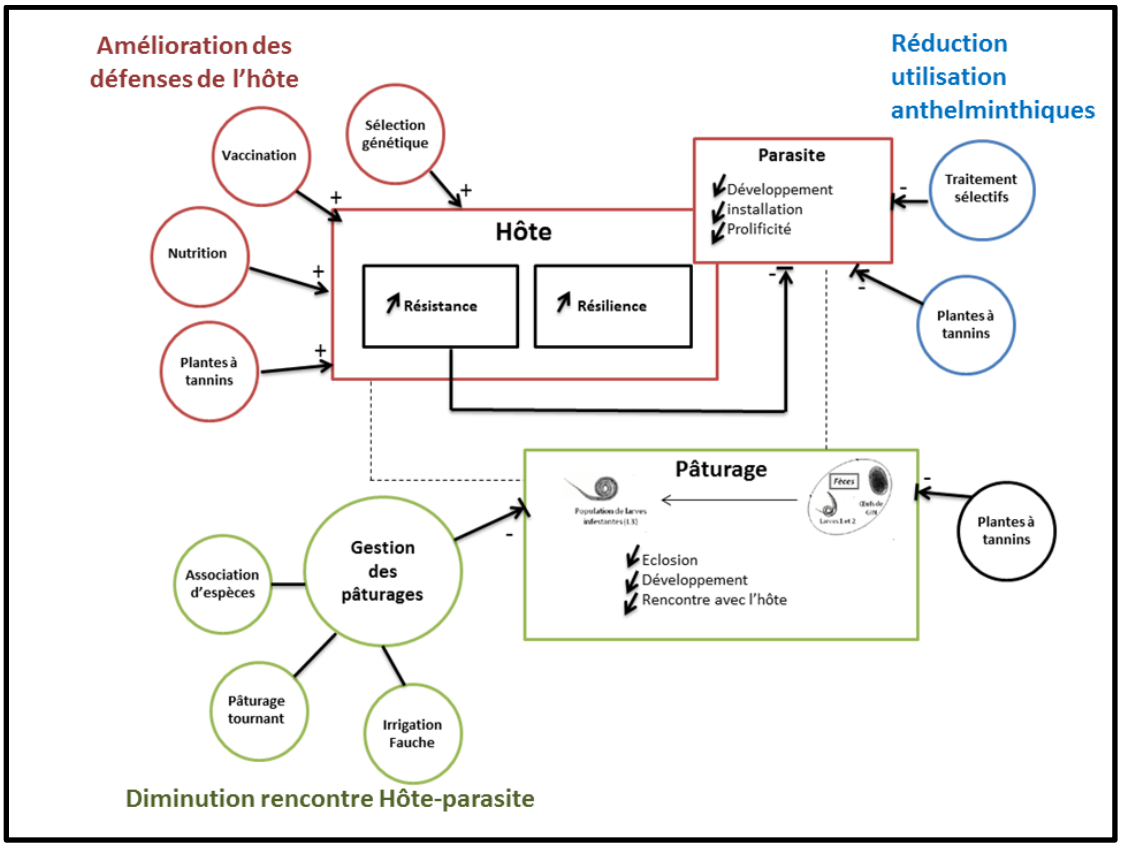


**Figure 1. Schéma du système de lutte intégrée (adapté de Mahieu et al., 2009)**

Les pathologies liées aux SGI sont présentées par certains auteurs comme une maladie nutritionnelle. En effet, l'alimentation apporte des nutriments qui impactent les fonctions immunitaires ; or, les SGI peuvent induire une réduction de l'ingestion et de la digestibilité des aliments. Certains auteurs suggèrent même qu'il pourrait s'opérer une réorientation de l'utilisation des nutriments pour lutter contre l'infestation et réparer les tissus endommagés plutôt que de les mobiliser pour la production (Hoste et al., 2005). Des synthèses de la littérature ont été réalisées sur les interactions nutrition-parasitisme chez les ovins (Coop and Kyriazakis, 1999; Coop and Kyriazakis, 2001; Van Houtert and Sykes, 1996; Walkden-Brown and P. Kahn, 2002). Elles montrent des interactions entre le statut nutritionnel des animaux et leurs résilience / résistance vis-à-vis des SGI. Moins de travaux ont été réalisés chez les caprins (Bambou et al., 2011; Hoste et al., 2005). Ces travaux, qui restent très descriptifs, montrent une augmentation de la réponse immunitaire antiparasitaire inflammatoire (hyper-éosinophilie) et à médiation humorale (Immunoglobulines A, E et G1). Cependant les mécanismes sous-jacents ne sont pas encore clairement identifiés (Hoste et al., 2005; Houdijk, 2012). L'effet positif de supplémentation en protéines dans la mise en place de réponses de résistance et de résilience est démontré mais reste variable. De plus, l'effet de l'énergie n'est pas clairement établi.

## 2. Bases et état des connaissances sur les interactions nutrition et parasitisme

### 2.1. Diversités des parasites

Les parasites gastro-intestinaux peuvent être des métazoaires ou des protozoaires. Parmi les métazoaires, on retrouve les helminthes avec principalement deux grands groupes : les nématodes et les plathelminthes. Les helminthes sont présents dans le monde entier (cf. Tableau 1) avec une forte prévalence en zone intertropicale. Les protozoaires qui affectent les élevages sont principalement du genre *Eimeria*. Le Tableau 2 liste les principaux helminthes pathogènes gastro-intestinaux. En Guadeloupe, terrain d'étude du projet, on rencontre une vingtaine de strongles digestifs dans les élevages dont *Haemonchus Contortus, Trichostrongylus colubriformis, et Oesophagostomum columbianum* (Aumont 1997). La plupart des SGI infestant les petits ruminants ont un cycle de vie monoxène. Ce cycle est composé d'une phase externe (pâturage) et d'une phase interne (hôte). Les œufs excrétés via les fèces d'animaux infestés éclosent et passent par plusieurs stades de développement larvaire. Après être passées par les stades L1 et L2 (stades larvaires 1 et 2), les larves vont atteindre le stade infestant L3 quelques jours après l'éclosion. Après ingestion des larves infestantes par l'hôte, elles vont migrer vers la muqueuse intestinale cible. Après une

succession de mues en stade L4 puis L5, les larves ont atteint le stade adulte. Les jeunes vers adultes vont évoluer et devenir sexuellement actifs pour atteindre le stade adulte mature (cf. Figure 2.).

**Tableau 1. Exemples de strongles gastro-intestinaux du petit ruminant**

| Reference | Région/ Organe | *Haemonchus* | *Trichostrongylus* | *Oesophagostomum* | *Cotylophoron* | *Mecistocirrus* | *Teladorsagia* | *Strongyloides* | *Cooperia* | *Bunostomum* | *Moniezia* | *Thysaniezia* | *Avitellina* | *Trichuris* | *Skrjabinema* | *Nematodious* | *Autres* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Abomasum | Intestin grêle/gros | Gros intestin | Rumen | Abomasum | Abomasum | Intestin grêle | Intestin grêle | Intestin grêle | Intestin grêle | Intestin grêle | Intestin grêle | Gros intestin | Gros intestin | Intestin grêle | |
| (Aumont 1997) | Guadeloupe | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | |
| (Crook et al., 2016) | USA | X | X | | | | X | | | | | | | | | X | |
| (Zainalabidin et al., 2015) | Malaisie | X | | | | | | | | | | | | | | | |
| (A.M.G. Belem, 2005) | Afrique | X | X | X | | | | X | | | | | X | X | X | | X |
| (Kearney et al., 2016) | Australie | X | | | | | | | | | | | | | | | |
| (Herrera-Manzanilla et al., 2017) | Mexique | X | X | X | | | | | | | | | | | | | |
| (Morgan et al., 2007) | Kazakhstan | X | | | | | | | | | | | | | | X | X |
| (Di Loria et al., 2009) | Italie | X | X | X | | | | | | | | | | | | | |
| (Cériac et al., 2017) | Guadeloupe | X | | | | | | | | | | | | | | | |
| (Matos et al., 2017) | Brésil | X | X | X | | | | | | | | | | | | | |
| (Nisbet et al., 2013) | Royaume-Unie | | | | | | X | | | | | | | | | | |
| (Piedrafita et al., 2012) | Australie | X | | | | | | | | | | | | | | | |
| (Arsenos et al., 2007) | Grèce | X | X | X | | X | | | X | X | | | X | | | X | |
| (Arece-García et al., 2017) | Cuba | X | X | X | | | | | | | | | | | | | |
| (Chiejina and Behnke, 2011) | Afrique | X | X | X | | | | X | X | | | | | | | | |
| (Domke et al., 2013) | Norvège | X | X | X | | | X | | X | X | | | | X | X | | X |
| (Sultan et al., 2016) | Egypte | | | | | | | X | | | X | | | | | X | X |
| (Miller et al., 2011) | USA | X | X | X | | | | | | | | | | | | | |

**Tableau 2. Principales espèces de Trichostrongles parasites des ruminants**

| Sous-famille | Espèces | Localisation chez l'hôte | Hôtes |
|---|---|---|---|
| Haemonchinae | ***Haemonchus contortus*** | **Caillette** | **Ovins, caprins** |
| | *Haemonchus placei* | Caillette | Bovins |
| | *Haemonchus similis* | Caillette | Bovins |
| | *Haemonchus longistipes* | Caillette | Dromadaires |
| Trichostrongylinae | ***Trichostrongylus colubriformis*** | **Intestin grêle** | **Ovins, caprins** |
| | *Trichostrongylus axei* | Caillette | Bovins, ovins, caprins |
| Ostertagiinae | *Teladorsagia circumcincta* | Caillette | Ovins |
| | *Ostertagia ostertagi* | Caillette | Bovins (ovins, caprins) |
| Cooperiinae | *Cooperia curticei* | Intestin grêle | Bovins, ovins, caprins |
| | *Cooperia oncophora* | Intestin grêle | Bovin, ovins |

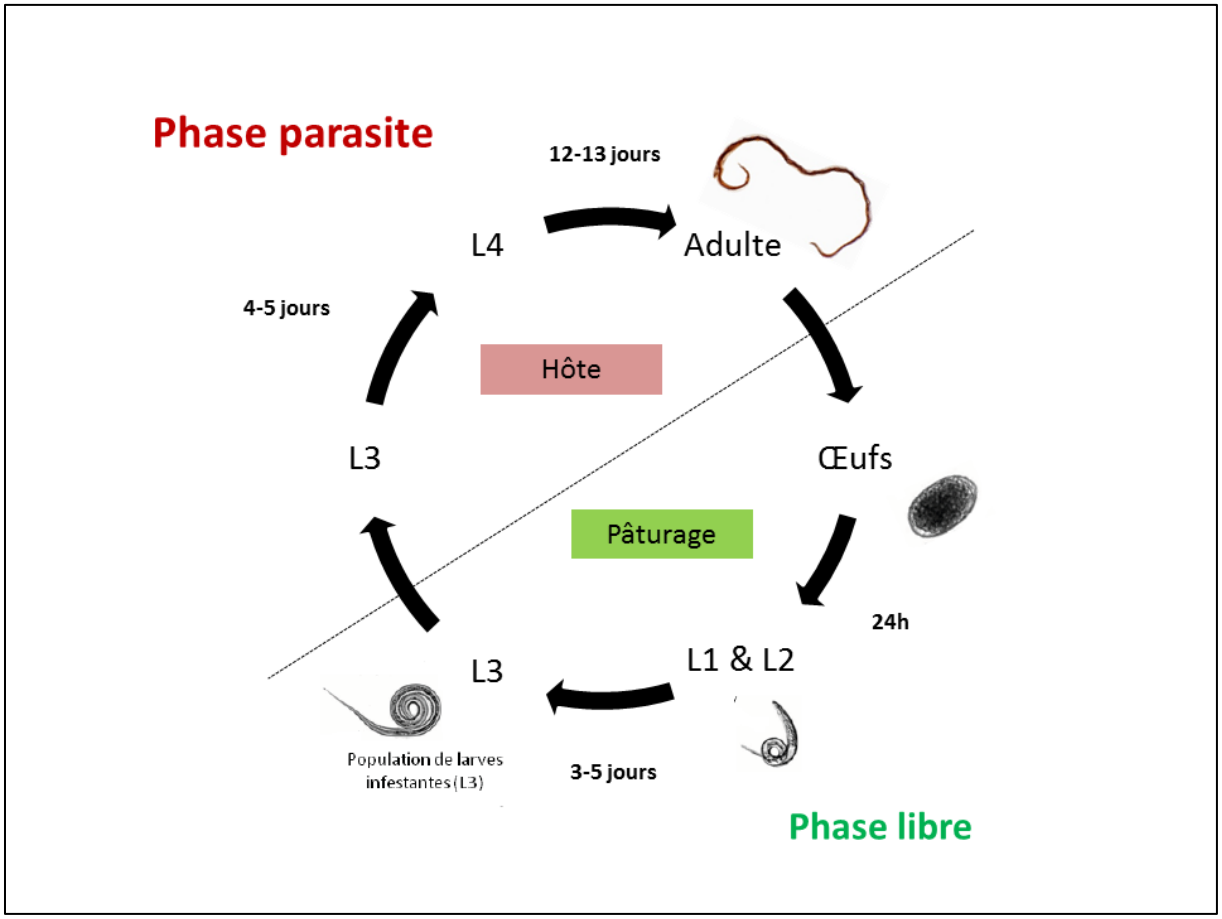


**Figure 2. Cycle de développement des Strongles gastro-intestinaux (BAMBOU, 2015)**

- *Haemonchus contortus*

Le parasite *Haemonchus contortus* a la plus forte prévalence dans les élevages dans le monde (Kearney et al., 2016). Ce nématode hématophage qui impacte sévèrement la santé des animaux est le modèle d'étude de nos travaux. C'est un parasite qui achève son cycle de développement dans la muqueuse de l'abomasum de son hôte. Après leur ingestion, les L3 perdent leurs gaines dans le rumen, puis pénètrent la muqueuse de l'abomasum pour muer en L4 entre le 4ème et 5ème jour d'infestation. Entre le 9ème et 11ème jour, les L4 muent en L5 ou adultes immatures (cf Tableau 3). Les mâles adultes mesurent entre 18 et 20 mm, alors que les femelles peuvent atteindre jusqu'à 30 mm. Ces dernières sont facilement reconnaissables par leur utérus enroulé autour de leur intestin rempli de sang, ce qui leur vaut le surnom de *Barber Pole worm* en anglais. Les premiers vers atteindront le stade adulte entre le 15$^{ème}$ et le 18$^{ème}$ jour d'infestation. Les premiers œufs seront excrétés dans les fèces après environ 3 semaines d'infestation, c'est la période prépatente.

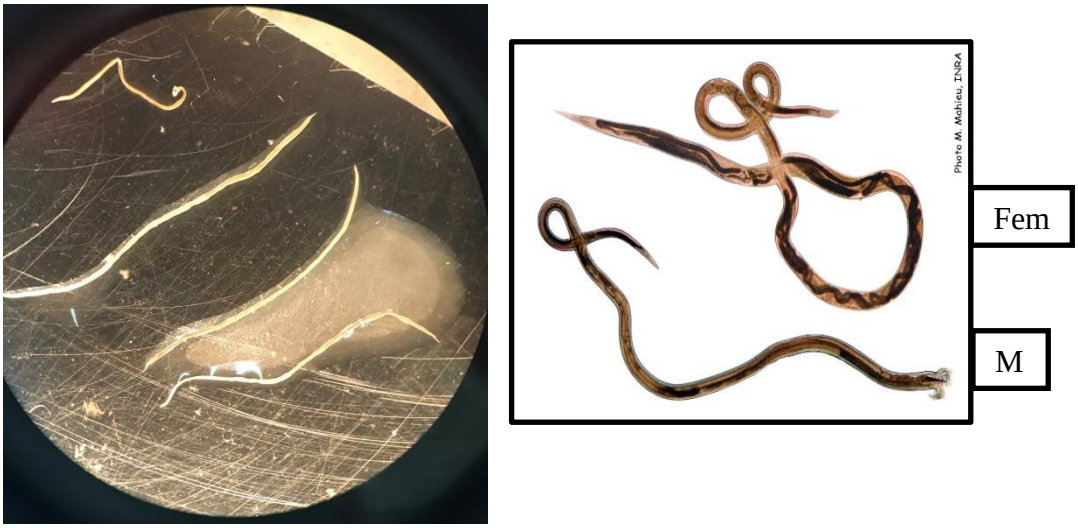


**Photo 1. Vers *H.contortus* mâle et femelle conservés dans du formaldéhyde**

- *Trichostrongylus colubriformis*

*Trichostrongylus colubriformis* est un nématode également présent dans de nombreuses régions du monde. Il s'installe dans l'intestin grêle. Au stade adulte *T. colubriformis* mesure entre 5 et 10mm. Il a une forme allongée et est de couleur brunâtre. Il possède un système digestif tubulaire, qui le traverse de la bouche à l'anus. Comme *H. contortus*, les mâles et femelles adultes matures sont facilement différenciables. Toutes les espèces de *Trichostrongylus* ont un cycle de développement direct. Les ruminants sont infestés par l'ingestion des L3. Ces dernières peuvent survivre jusqu'à 6 mois dans l'environnement.

Après leur ingestion, les L3 vont arriver dans l'intestin grêle entre 2 et 5 jours. Ces L3 vont perdent leurs gaines dans la caillette, puis pénétrer les muqueuses de l'intestin grêle pour se transformer en L4 entre le 7ème et 8ème jour d'infestation. Le 15ème jour, les L4 muent en L5. Les premiers vers atteindront le stade adulte 3 semaines après l'infestation (cf Tableau 3). *T. colubriformis* parasite surtout la partie antérieure de l'intestin grêle en entrainant parfois des pertes partielles de microvillosités du duodénum.

- *Oesophagostomum columbianum*

Le parasite *Oesophagostonum columbianum* est l'un des principaux nématodes parasitant les ruminants à travers le monde. Il peut aussi infester les porcs et les primates. C'est un vers rond hématophage dont les femelles adultes mesurent jusqu'à 24 mm de long, alors que les mâles mesurent jusqu'à 16 mm. Après leur ingestion, les L3 perdent leurs gaines dans l'intestin grêle, puis pénètrent les muqueuses de l'intestin grêle pour se muer en L4 dès le 10ème jour d'infestation. Le 15ème jour, les L4 muent en L5. Les premiers vers atteindront le stade adulte à partir du 20ème jour d'infestation (cf Tableau 3). C'est un nématode qui est associé à la formation de nodules dans le tractus gastro- intestinal de l'hôte qui va altérer l'absorption intestinale. Ces affections peuvent être responsables de sévères dysenteries, et peuvent être accompagnées de diminution d'appétit.

**Tableau 3. Les principales phases d'infestation des SGI infestant les petits ruminants**

| | ***Haemonchus contortus*** | ***Trischostrongylus colubriformis*** | ***Oesophagostomum columbianum*** |
|---|---|---|---|
| Présence des larves L3 dans l'organe cible après ingestion | 2ème jour | 2ème- 5ème jours | 2ème- 5ème jours |
| Mue des larves L3 en larves L4 | 4ème- 5ème jours | 7ème- 8ème jours | 10ème jour |
| Evolution des larves L4 en stades 5 juvéniles | 9ème- 11ème jours | 15ème jour | 15ème |
| Apparition des premiers stades adultes | 18ème jour | 20ème jour | 20ème jours |
| Excrétion des premiers œufs dans les fèces | 21ème jours | 21ème | 35ème jours |

## 2.2. Réponses animales à l'infestation par des SGI hématophages

### 2.2.1. Indicateurs d'infestation

#### 2.2.1.1. Excrétion d'œufs

L'excrétion d'œufs dans les fèces appelée OPG pour œufs par gramme, est un indicateur du niveau d'infestation des animaux par les SGI. Cette mesure d'OPG reste le paramètre le plus utilisé pour objectiver le niveau d'infestation notamment dans les schémas de sélection. Son héritabilité varie entre 0.3 et 0.4 chez les ovins et 0.15 et 0.2 chez les caprins (Chiejina and Behnke, 2011; Costa et al., 2000; Mandonnet et al., 2001; Rout et al., 2011; Safari et al., 2006). Néanmoins, le fait qu'il n'existe pas à ce jour de méthode automatisée de comptage et la difficulté de conserver les matières fécales sur de longues durées en font une technique lourde à mettre en place sur de grands effectifs.

Dans une méta-analyse récente, Ceï et al. (2018) quantifient une réduction de l'hématocrite de 5,81% et de 2,64 % chez les ovins et les caprins respectivement pour une augmentation de 1 point du Log d'OPG (Figure 3.). Cependant, dans cette même étude les auteurs indiquaient que les différences entre espèces animales disparaissaient quand l'ingestion d'énergie et de protéines étaient similaires.

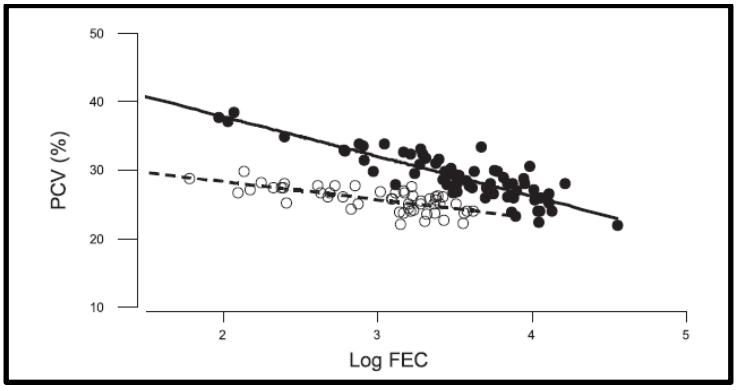


**Figure 3. Relation entre l'excrétion d'oeufs et l'hématocrite (PCV) chez des caprins et ovins. (Ceï et al., 2018).** *Les cercles et la droite pleine pour les ovins. Les cercles vides et la droite en pointillés pour les caprins.*

#### 2.2.1.2. Hématocrite

Les infestations gastro-intestinales sont souvent responsables d'anémie (Cériac et al., 2017; Kaplan et al., 2004). L'anémie est directement liée à l'activité hématophage des parasites mais aussi en partie à leur activité destructrice ou desquamative. Lorsque les parasites s'installent dans la muqueuse digestive, ils vont causer des lésions qui seront responsables d'hémorragie dans la lumière intestinale (Bown et al., 1991; Poppi et al., 1986). Une des

caractéristiques des infestations par *H. contortus* est la corrélation négative entre l'hématocrite des animaux et le niveau d'infestation mesuré via l'OPG (Barger and Dash, 1987; Kaplan et al., 2004; Lejambre et al., 1971; Mederos et al., 2014). Chez la chèvre Créole, une corrélation génétique de -0.35 a été mise en évidence entre hématocrite et OPG (de la Chevrotière, 2011b). Ce paramètre est d'ailleurs mesuré dans de nombreux schémas de sélection d'animaux résistants aux infestations par les SGI notamment chez la chèvre Créole (Gunia, 2012 ). Des QTL de résistance aux SGI ont été mis en évidence sur ce caractère (de la Chevrotière, 2011a).

#### 2.2.1.3. Les polynucléaires éosinophiles

Les polynucléaires éosinophiles tissulaires et circulants sont des marqueurs d'infestation parasitaire, en particulier du tube digestif (Schallig, 2000). La réaction des éosinophiles contre une infestation parasitaire n'est pas spécifique. Les éosinophiles sont des granulocytes qui s'attaquent aux stades larvaires des parasites notamment en libérant le contenu de leurs granules (Huang and Appleton, 2016; Meeusen and Balic, 2000). Ils peuvent passer du sang vers les tissus afin d'interagir directement avec les parasites. Les éosinophiles sont attirés par chimiotactisme dans les zones d'inflammation par les mastocytes et les basophiles. Des études ont suggéré que cette population cellulaire jouait un rôle clé dans la résistance génétique aux SGI puisqu'une corrélation entre les niveaux d'infestation et l'intensité de l'éosinophilie sanguine avait été observée (Meeusen et al., 2005). Une étude *in vivo* a montré que les éosinophiles peuvent interagir directement avec les formes larvaires des SGI et endommager les NGI (Balic et al., 2006; Robinson et al., 2010). Cette capacité à s'attaquer aux larves de SGI a également été démontrée *in vitro* (Terefe et al., 2007). Cette réponse n'est pas observée dans toutes les études, notamment dans les études avec *T. circumcincta* (Beraldi et al., 2008; Henderson and Stear, 2006). Cette différence serait probablement due au fait que *H. contortus* induit une inflammation tissulaire beaucoup plus marquée que *T. circumcincta* (Venturina et al., 2013). Par ailleurs, les travaux conduits sur la chèvre Créole en Guadeloupe ont montré qu'il y avait une hyper éosinophilie sanguine chez les animaux infestés par *H. contortus* mais aucune corrélation n'a été mise en évidence avec la résistance génétique (Bambou et al., 2008; Bambou et al., 2009; Bambou et al., 2013). Il semble donc que le rôle des éosinophiles dans la réponse immunitaire contre les SGI ne soit pas identique chez les ovins et les caprins. Des travaux montrent que l'éosinophilie sanguine est inversement corrélée aux OPG chez les ovins (Buddle et al., 1992; González et al., 2011; Rothwell et al., 1993). Chez les caprins, la situation est inversée, les OPG sont positivement corrélés à l'éosinophilie sanguine (Bambou

et al., 2009). Bien qu'une diminution des éosinophiles circulants puisse être le résultat d'une infiltration tissulaire importante, il n'a pas été mis en évidence de corrélation entre l'éosinophilie sanguine et tissulaire. Néanmoins, chez les ovins et les caprins, l'éosinophilie sanguine est un caractère faiblement mais significativement héritable ($h^2$=0.2, (de la Chevrotière, 2011a)).

### 2.2.2. La réponse immunitaire contre les strongles gastro-intestinaux

Le système immunitaire a une composante innée (la réponse innée non spécifique) et une composante acquise (la réponse adaptative spécifique) liées par l'intermédiaire de cellules et de molécules de signalisation. La composante innée, la première ligne de défense après l'entrée du parasite est constituée : d'une barrière physique (e.g. la muqueuse du tube digestif, le mucus); d'une barrière cellulaire (e.g. les phagocytes mononucléaires, monocytes et macrophages; les cellules dendritiques; les granulocytes polymorphonucléaires, neutrophiles, éosinophiles et basophiles). C'est une réponse rapide qui met en place des mécanismes non spécifiques. La réponse adaptive, qui se développe au cours de la vie de l'hôte, est composée de la réponse humorale et la réponse cellulaire.

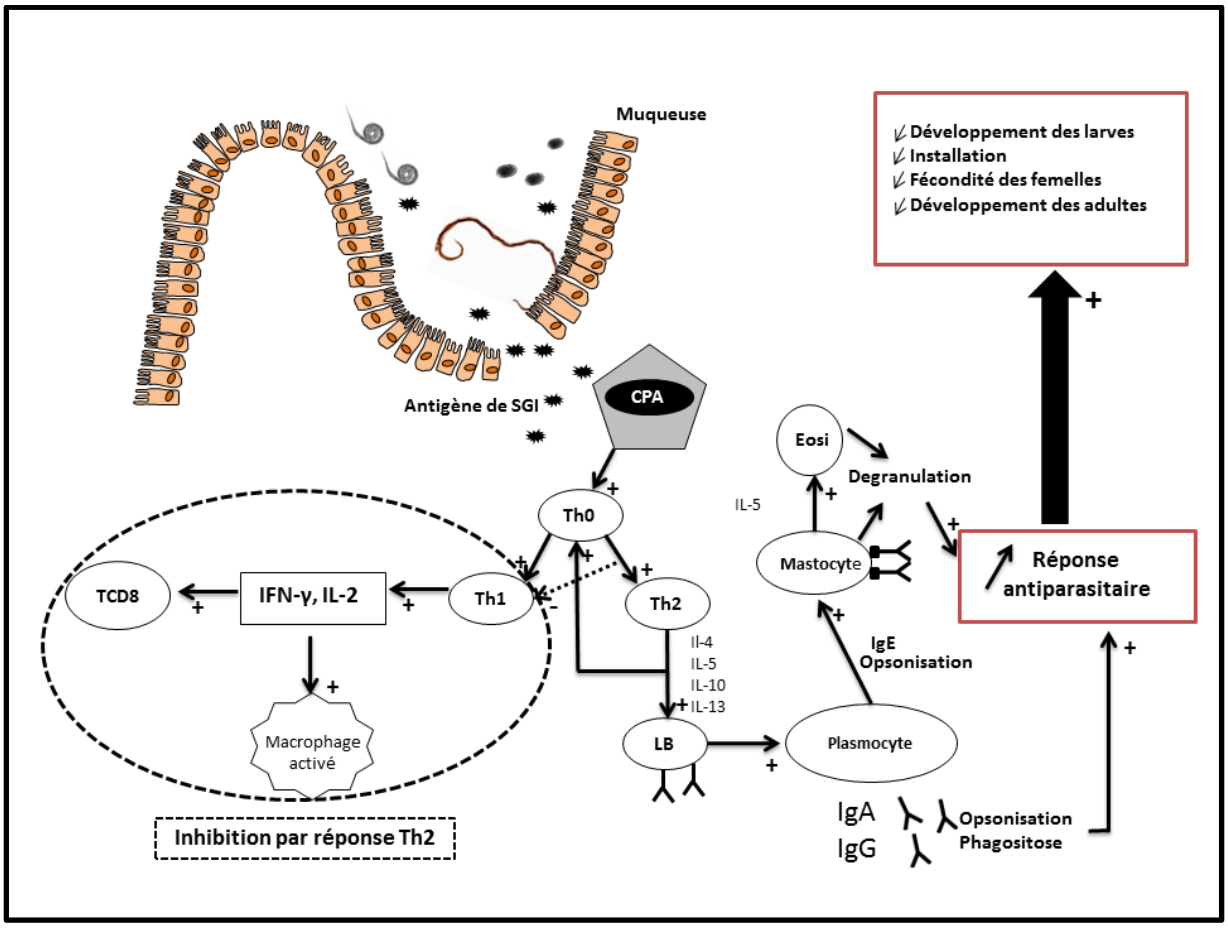


**Figure 4. Représentation schématique de la réponse immunitaire contre les NGI. (Adapté de Bambou, 2015)**

### 2.2.2.1. La réponse cellulaire

La réponse immunitaire à médiation cellulaire est initiée par les cellules présentatrices d'antigènes (CPA) comme les macrophages, les cellules dendritiques ou les lymphocytes B. C'est l'interaction entre le complexe antigène-CPA et les récepteurs TCR (T-cell receptor) exprimés par les lymphocytes TCD4+ naïfs qui active la différentiation de ces derniers en lymphocytes T4 effecteurs. Dans les modèles bien caractérisés tels que la souris ou l'humain, les lymphocytes T4 effecteurs se différencient en lymphocytes T-helper Th1 ou Th2 selon que le pathogène soit intracellulaire ou extracellulaire (Harris et al., 2009; Mak et al., 2014; Urban Joseph et al., 1992). Il faut noter que chez les ruminants, l'existence d'une dichotomie Th1/Th2 n'est pas totalement démontrée, en partie à cause du manque d'outils (Hope et al., 2012). La réponse Th2 inhibe la Th1 et réciproquement. Les lymphocytes Th1 activent les macrophages par l'intermédiaire de cytokines comme l'interleukine 2 (IL-2) ou l'interféron γ (IFN-γ). La réponse Th2 serait associée à la résistance génétique contre les SGI (Gill et al.,

2000; Jacobs et al., 2015; Lacroux et al., 2006; Wilkie et al., 2015) (Figure 4.). Les lymphocytes Th2 produisent principalement des cytokines IL-4, IL-5, IL-10 et IL-13 qui activent la différentiation des lymphocytes B (LB) en plasmocytes qui produisent des immunoglobulines IgA, IgG, IgE (Miller and Horohov, 2006; Neurath et al., 2002). La réponse Th2 est également associée à la production de granulocytes éosinophiles sanguins qui ont un rôle connu dans la réponse contre les SGI (cf. partie 2.2.1.3). De la même manière, les mastocytes intra-épithéliaux (globules leucocytes) seraient impliqués dans la réponse contre les formes larvaires de SGI à la fois chez les ovins et les caprins (Balic et al., 2002; Bambou et al., 2013; Huntley et al., 1992; Kemp et al., 2009; Robinson et al., 2010) .

#### 2.2.2.2. La réponse humorale

Chez les ovins, la réponse humorale médiée, notamment par les immunoglobulines A et E (IgA et IgE) joue un rôle clé dans la réponse protectrice contre les SGI. Les IgA sont associées à une diminution du taux d'installation des larves et de la fécondité des femelles par des mécanismes qui restent à élucider (Stear et al., 1995; Stear et al., 2009; Stear et al., 1999; Strain and Stear, 2001). La réponse IgE est également associée à une diminution de l'installation des L3 et L4 (Huntley et al., 2001; Murphy et al., 2010). Le mécanisme responsable serait une réponse d'hypersensibilité de type 1 avec une dégranulation des mastocytes résultant de l'interaction des antigènes des SGI avec les IgE fixées sur les mastocytes (Huntley et al., 1992; Stear et al., 2003). Chez les caprins, la réponse humorale est peu étudiée hormis chez les chèvres Créole de Guadeloupe. Des corrélations génétiques élevées entre la réponse en IgA et les OPG ont été mises en évidence (r=0.84 et 0.72 pour les IgA dirigées contre les adultes et contre les L3, respectivement) et une corrélation génétique modérée ($h^2$= -0.32) a été mise en évidence entre la réponse en IgE et les OPG (Bambou et al., 2008). Les IgA et IgE seraient donc impliquées dans des mécanismes clés de la résistance aux SGI chez les caprins Créole.

### 2.2.3. Ingestion et digestion des aliments

La littérature témoigne d'effets variables des SGI sur l'ingestion et la digestion des rations. Une revue rapporte des réductions d'ingestion de 6 à 30% et des réductions de digestibilité pouvant atteindre jusqu'à 40% (Poppi et al., 1990). Cependant, la tendance générale serait une réduction moyenne de la matière sèche volontairement ingérée de 8.7 g/kg du poids métabolique (poids vifs élevé à la puissance 0.75, $PV^{0.75}$) par point du log de l'OPG, sans différence significative entre ovins et caprins (Ceï et al., 2018). La même tendance est

observée pour la digestibilité de la matière organique qui décroit en moyenne de 1,2% par point du log de l'OPG (Ceï et al., 2018). Dakkak (1995) rapporte des différences de 25% pour la digestibilité de la matière organique et de la protéine brute entre animaux infestés et non-infestés. La variabilité des résultats rapportés dans la littérature pourrait être liée à la quantité de larves consommées et à l'espèce de SGI. Les strongles de l'abomasum (*H. contortus ; O. circumcincta ; T. axei*) auraient un effet plus marqué sur la réduction de l'ingestion que les strongles de l'intestin (*Trichostronglyus*, *Cooperia* et *Oesophagostomum*). L'intensité de l'anorexie pourrait également varier avec le statut immunitaire de l'animal. Elle serait plus marquée chez les jeunes animaux naïfs en phase d'activation de leur système immunitaire et disparaitrait à l'âge adulte (Sykes, 2010). La réduction moyenne de l'ingestion pendant la phase d'activation du système immunitaire serait de 40% chez les jeunes contre moins de 5% chez les animaux adultes. Les différences de digestibilité ont été expliquées par une hyper motricité du tube digestif avec pour conséquence une réduction du temps de transit des aliments (Bueno et al., 1982).

### 2.2.4. Croissance et surcout nutritionnel du parasitisme

Le parasitisme gastro-intestinal impacte négativement la croissance des petits ruminants avec un effet plus marqué chez les caprins comparativement aux ovins. Ainsi, le gain moyen quotidien de poids décroît respectivement de 1,55 et de 4,55 g/kg $PV^{0,75}$/unité de log de l'OPG (Ceï et al., 2018) chez les ovins et les caprins. Ce résultat s'explique davantage par le coût nutritionnel supplémentaire lié au parasitisme que par la baisse de l'ingestion et de la digestibilité qui peuvent aussi être observées. Ce surcoût nutritionnel est lié à la fois aux effets physiopathologiques directs des SGI et à l'activité de la fonction immunitaire. En effet, la mise en place d'une réponse immunitaire est coûteuse en terme de protéines et d'énergie pour satisfaire les besoins métaboliques des cellules immunitaires et permettre la synthèse des médiateurs protéiques de l'immunité et la réparation des lésions tissulaires (Liu et al., 2005; Lochmiller and Deerenberg, 2000).

### 2.2.5. Différences entre ovins et caprins et variabilité génétique

Les ovins et caprins peuvent être classés en animaux sensibles, résistants et résilients aux infestations par les SGI. La résistance génétique aux SGI est la capacité de l'animal à limiter l'installation et par conséquent le niveau d'infestation. La résilience est la capacité à limiter les effets physiopathologiques des SGI et par conséquent à maintenir un certain niveau de production, contrairement aux animaux sensibles. Les animaux résilients ont la capacité

d'atténuer les effets délétères du parasitisme sans pour autant limiter le niveau d'infestation. La sensibilité est l'incapacité à contrôler le niveau d'infestation et ses effets délétères et peut conduire à la mort de l'animal en l'absence d'un traitement anthelminthique efficace. La résistance, la résilience et la sensibilité des animaux sont influencées essentiellement par leur stade physiologique, leur alimentation et leur génotype. Ainsi, les jeunes animaux en croissance qui n'ont pas encore de système immunitaire mature et les mères en fin de gestation qui subissent une dépression physiologique de leur système immunitaire sont les plus vulnérables à ces infestations. Il est généralement admis que les caprins sont plus sensibles aux infestations par les SGI que les ovins. Ainsi des auteurs rapportent que l'excrétion fécale d'œufs serait plus élevée chez le caprin comparativement à l'ovin consommant la même prairie (Hoste et al., 2008). Cette différence serait due à une réponse immunitaire des caprins moins efficace que celle des ovins (Bambou et al., 2009; Hoste and Torres-Acosta, 2011).

## 3. Effet de la stratégie alimentaire contre les strongles gastro-intestinaux

### 3.1. De l'aliment aux nutriments

Chez les ruminants, les nutriments ont 3 origines principales : la digestion des aliments, la synthèse de populations microbiennes gastro-intestinales et la mobilisation des réserves. Les Figure 5. et Figure 6. résument les principales caractéristiques de la digestion chez les ruminants.

La quantité et la qualité des nutriments pour chacune des origines sont aussi variables. Archimede et al., (1997) ont quantifié les lois de réponses et les principaux facteurs de variations de la digestion de la matière organique, des glucides cellulaires et pariétaux, et de la synthèse microbienne chez les ruminants. Ces auteurs rapportent une large variabilité de la partition de la digestion entre le rumen et l'intestin. Ils ont estimé la contribution du rumen à la digestion totale de la matière organique, les parois végétales et l'amidon à respectivement 66,7%±8,7, 78,8%±8,1 et 80,5%±16,3. En conséquence, les principaux nutriments issus de la digestion varient en quantité et qualité.

#### 3.1.1. Les Acides Gras Volatils (AGV)

Les AGV sont les principaux nutriments énergétiques de la digestion de la matière organique dans le rumen. Ces AGV sont composés principalement d'acétate, de propionate et de butyrate. Le propionate est le principal précurseur de la synthèse de glucose via la néoglucogenèse. Ce nutriment est particulièrement important chez le ruminant qui comparativement aux monogastriques non herbivores, absorbe relativement peu de glucose du fait des caractéristiques de sa physiologie digestive et de son alimentation. La digestion des fourrages, base de l'alimentation du ruminant, conduit généralement à des profils d'AGV relativement riches en acétate et pauvres en propionate, comparativement aux rations mixtes contenant des ingrédients amylacés. Par contre, la digestion de jeunes fourrages (moins de 28 jours) produit plus de propionate que celle de fourrages plus âgés. La digestion ruminale de l'amidon est donc le principal facteur de variation de la production de propionate. Cependant, la nature de l'amidon va conditionner l'importance de sa digestion dans le rumen. Ainsi, Sauvant et al., (1994) rapportent des digestions ruminales comprises entre 90 et 95 % pour les amidons à dégradation rapide de certains aliments (e.g. blé, orge) contre 50 à 90% pour les amidons à dégradation lente (e.g. maïs, riz, sorgho). La plus faible digestion ruminale de l'amidon est compensée par une digestion intestinale plus élevée avec une production et une absorption plus importante de glucose. Toutefois, à notre connaissance, la démonstration

d'une plus grande disponibilité de glucose sanguin n'a pas été démontrée à ce jour du fait de sa forte consommation par la paroi intestinale.

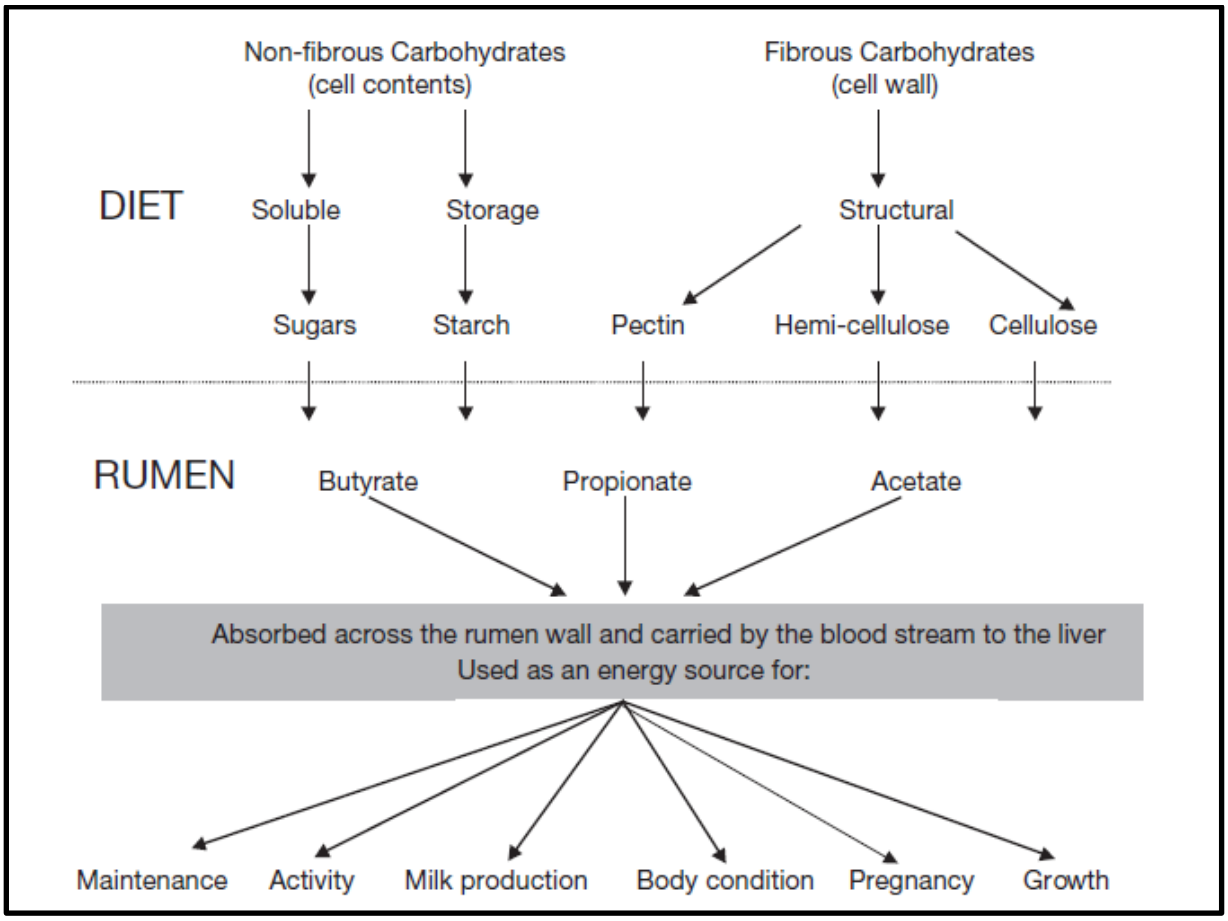


**Figure 5. Métabolisme des glucides du ruminant (Moran, 2005)**

### 3.1.2. Les Acides Aminés

La quantité et la qualité des AA absorbés dans l'intestin dépendent de la composition en protéines de l'aliment, de leur dégradation microbienne dans le rumen et de la synthèse de protéines microbiennes dans le rumen. En fonction de leurs caractéristiques, des proportions variables de protéines alimentaires sont dégradées dans le rumen. Une grande variabilité de la synthèse ruminale de protéines microbiennes est observée dans la littérature, avec une moyenne estimée de 23,5 g/kg de matière organique dégradée dans le rumen et un écart type de ± 9,3 (Archimede et al., 1997). Quand les ruminants consomment des rations mixtes (fourrage et concentré), l'efficacité de la synthèse microbienne est optimale lorsque la part de concentré est de l'ordre de 40 % de la ration (Archimede et al., 1997). La nature des glucides impacte l'efficacité de la synthèse microbienne. Cette dernière est plus élevée avec l'amidon lentement dégradable et les parois dégradables (+ 6,6 g d'azote microbien / kg de matière

organique réellement fermentée dans le rumen) comparativement à l'amidon rapidement dégradable. Cependant, la réduction de la digestion ruminale de l'amidon réduit la protéosynthèse microbienne dans le rumen et le flux intestinal de protéines microbiennes (-9,3 g/ 100 g d'amidon protégé) (Sauvant et al., 1994).

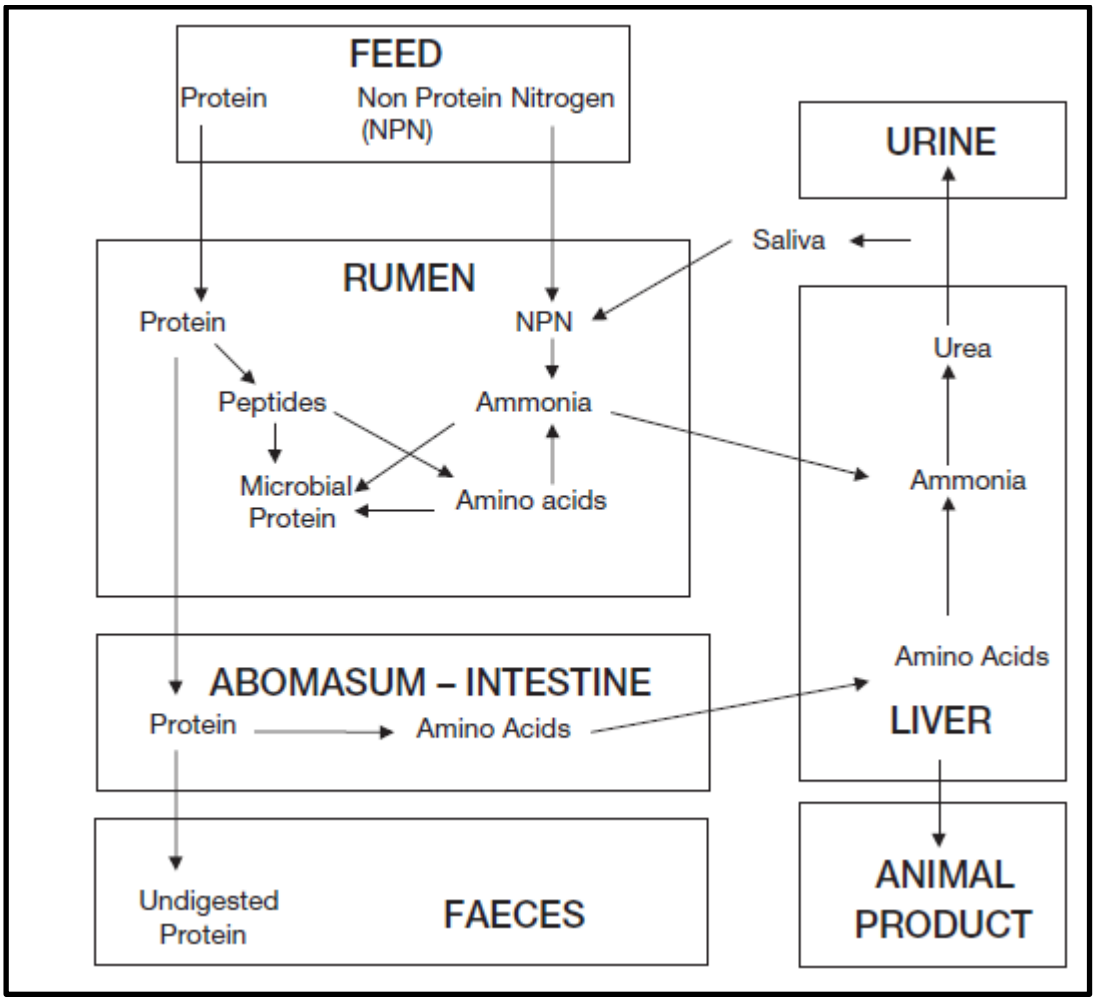


**Figure 6. Métabolisme de l'azote chez le ruminant (Moran, 2005)**

### 3.1.3. Les profils d'acides aminés

Comme toutes les autres espèces, les ruminants synthétisent les protéines qui jouent un rôle structural ou fonctionnel à partir des AA. Chez les ruminants, contrairement aux monogastriques, les protéines qui arrivent à l'intestin sont très différentes de celles apportées par l'alimentation. En effet, c'est un mélange de protéines alimentaires qui ont échappé à la dégradation de la flore microbienne du rumen et de protéines fabriquées par la flore microbienne du rumen à partir des AA des protéines alimentaires, de fractions azotées non protéiques et des protéines endogènes produites par le système digestif. Le profil des AA

d'origine microbienne est relativement stable comparativement à celui provenant des aliments. La proportion de ces différentes fractions est directement liée à la composition de la ration. Les protéines microbiennes et endogènes représentent respectivement en moyenne plus de 50% et jusqu'à 15% de la fraction duodénale (Clark et al., 1992; Ouellet et al., 2002). Ainsi, chez les ruminants, l'intérêt de raisonner l'alimentation en termes d'AA de la ration fait encore débat. Certains auteurs considèrent que les protéines microbiennes sont suffisamment équilibrées pour satisfaire les besoins des animaux. D'autres auteurs pensent que la composition des protéines microbiennes n'est pas optimale en s'appuyant sur des travaux montrant que la lysine et la méthionine s'avèrent limitantes pour la production des protéines du lait, la croissance des jeunes bovins, ovins et caprins et la production de fibres (i.e. laine, cachemire) (Bocquier et al., 1994; Galbraith, 2000; Han et al., 1996; Klemesrud et al., 1997; Lynch et al., 1991; Matras et al., 2000; Muramatsu et al., 1993; Reis and Tunks, 1976; Rulquin, 1992; Rulquin and Champredon, 1987; Sahlu and Fernandez, 1992; Schwab, 2007; Titgemeyer et al., 1988).Bien que les AA soient préférentiellement utilisés pour la synthèse de protéines, certains AA, appelés AA glucoformateurs, peuvent contribuer jusqu'à 20% au métabolisme énergétique notamment via la néoglucogenèse.

A notre connaissance, il n'y a pas de travaux étudiant l'effet d'AA individuels sur la réponse des petits ruminants aux infestations par les SGI.

### 3.1.4. Les micronutriments

Les micronutriments (i.e. les vitamines et les minéraux inorganiques) présentent la caractéristique d'être indispensables en petite quantité (d'où le suffixe micro) à différentes fonctions dans l'organisme. En général, ils doivent être apportés par l'alimentation car l'organisme ne peut pas les synthétiser. Il est d'usage de présenter les minéraux en deux groupes, les macroéléments ou éléments majeurs et les éléments traces ou oligoéléments. Cette classification est fonction des besoins des animaux, des rôles et des fonctions qui sont différents entre les deux groupes. Le Tableau 4 donne une vision synthétique des principales fonctions physiologiques de 15 minéraux d'intérêt dans la nutrition des ruminants.

**Tableau 4. Les principales fonctions physiologiques des 15 éléments minéraux d'intérêt pour la nutrition des ruminants (INRA, 2018)**

| | Teneur dans l'organisme (/kg PV) | Fonctions principales |
|---|---|---|
| P | 10 g (70-80% dans les os) | Métabolisme osseux, fonction structurelle et métabolique. Composant du système tampon du sang, des parois cellulaires et des contenus cellulaires (phospholipides, acides nucléiques). Impliqué dans presque toutes les transactions énergétiques basées sur la rupture ou la formation d'ATP. Requis dans le rumen pour la digestion de la cellulose (recyclage de la salive). |
| Ca | 16 g (99% dans les os) | Métabolisme osseux, fonction structurelle et métabolique. Déclenche la contraction et la relaxation musculaire. Coagulation sanguine. Impliqué dans l'activité de nombreuses enzymes. Messager secondaire important pour la signalisation cellulaire. |
| Mg | 0,5 g (60-70% dans les os) | Cofacteur de nombreuses réactions enzymatiques impliquées dans toutes les voies métaboliques majeures. Magnésium extracellulaire vital pour la conduction nerveuse, la fonction musculaire. Composant des os et nécessaire pour la minéralisation osseuse. |
| K | 3 g (66% peau et muscles) | Impliqué dans le contrôle de la pression osmotique et la régulation acido-basique (cation intracellulaire principalement). Activateur ou cofacteur de nombreuses réactions enzymatiques régulant le métabolisme énergétique, l'absorption cellulaire des acides aminés et la synthèse des protéines. |
| Na | 1,7-2,0 g (40% dans les os) | Impliqué dans la régulation du volume de liquide extracellulaire et l'équilibre acido-basique (cation extracellulaire primaire). Rôle indispensable dans les pompes Na-K ATPase impliquées dans la régulation de l'absorption cellulaire du glucose, des AA et des phosphates et dans la sécrétion cellulaire des protons, Ca, bicarbonates, K et Cl. |
| Cl | 1,2-1,4 g | Impliqué avec le Na dans la régulation du volume de liquide extracellulaire et l'équilibre acido-basique (anion extracellulaire). Principal anion des sécrétions gastriques permettant la digestion des protéines et la solubilisation des phosphates. |
| S | 1,5-2,0 g | Composant de plusieurs acides aminés (méthionine, cystéine, cystéine, taurine), vitamines (thiamine et biotine) et hormones (insuline, ocytocine). Essentiel pour assurer une synthèse protéique maximale dans le rumen. Impliqué dans tous les principaux métabolismes. Rôle particulier dans la synthèse du cartilage et des phanères. |
| Co | 0.02 mg | Composant de la vitamine $B_{12}$ – impliqué dans l'activité cellulolytique ruminale. |
| Cu | 2 mg (os, muscle, foie) | Composant, cofacteur ou activateur de nombreuses enzymes impliquées dans le métabolisme et le transport du fer, la synthèse de myéline, du collagène et de l'élastine (os et cartilage), le système antioxydant, et l'implantation de l'embryon. |
| I | 0.4 mg (70-80% glande thyroïde) | Composant de l'hormone thyroïdienne (régulation de l'oxydation cellulaire, de la synthèse des protéines, métabolisme basal, du système immunitaire, thermorégulation, reproduction, développement fœtal). |
| Mn | 3 mg (foie, os, pancréas, reins) | Composant ou activateur des enzymes impliquées dans la synthèse du cartilage et de la substance fondamentale de l'os (protéine impliquée dans la structure osseuse), métabolisme glucidique et lipidique, système antioxydant, métabolisme des gonades. |
| Se | 0.2 mg (reins, foie) | Composant de plusieurs enzymes (sélénoprotéines), comme la glutathion peroxydase (protection cellulaire contre l'accumulation de peroxyde d'hydrogène) et déiodinase (convertit T4 et T3). Autres sélénoprotéines impliquées dans les métabolismes liés au développement foetal, à la reproduction et au système immunitaire. |
| Zn | 30 mg (peau & phanères) | Composant de nombreuses métalloenzymes qui affectent les métabolismes glucidiques, lipidiques, protéiques et celui des acides nucléiques. |
| Cr | < 0.1 mg | Implication dans le complexe moléculaire connu sous le nom de facteur de tolérance au glucose, qui potentialise l'effet de l'insuline pour la capture du glucose par les cellules. |
| Mo [1] | ≈ 0.01 – 0.05 mg | Composant de 3 enzymes (xanthine oxydase, sulfite oxydase et aldéhyde oxydase) impliquées dans les métabolismes protéique et lipidique. |

Les vitamines sont également présentées en deux groupes, les vitamines liposolubles et les vitamines hydrosolubles. Les vitamines liposolubles sont des composés hydrophobes dont l'absorption ne peut être efficace que si les lipides sont normalement absorbés. Elles sont transportées dans le sang sous forme de lipoprotéines ou attachées à des protéines de transport. Les vitamines hydrosolubles jouent essentiellement le rôle de cofacteurs enzymatiques. Le Tableau 6 donne une vision synthétique des principales fonctions biologiques et sources pour les ruminants de 13 vitamines. A la différence des acides aminés, l'impact de la supplémentation en micronutriments sur la réponse des petits ruminants aux infestations par les SGI a fait l'objet de nombreux travaux qui sont résumés dans le Tableau 5.

**Tableau 5. Effets des micronutriments la réponse des petits ruminants aux infestations. (↙, *diminution ; ↗, augmentation*)**

| Macronutriments | Dose | effet | Parasite | Référence |
|---|---|---|---|---|
| Phosphore (P) | Supplémentation | ↗ croissance, ↙ de la charge parasitaire, et ↙ de l'excrétion d'œufs | *T.vitrinus* | (Pathak, 2017) |
| Zinc (Zn) | Supplémentation | ↙ la réponse immunitaire Th2 (↙ la prolifération des lymphocytes T, ↙ production éosinophile, des IL-4, IgG, IgE) | | (Koski Kristine and Scott Marilyn, 2003) |
| Fer (Fe) | Supplémentation | Reconstitution du fer perdu par les pertes en GR | | (Pathak, 2017) |
| Cuivre (Cu) | Supplémentation | Impacte directement les NGI, ↗ réponse immunitaire ↙ Etablissement, l'excrétion d'œufs, le développement et fécondité des femelles | *T.circumcinacta , H.contortus* | (Chartier et al., 2000; de Montellano et al., 2007; Pathak, 2017) |
| Manganese (Mn) | Excès | ↗ l'infestation et nuisible à la réponse immunitaire | | (Pathak, 2017) |
| Cobalt (Co) | Carence | ↙ la résistance aux NGI | | (Pathak, 2017) |
| Sélénium (Se) | Supplémentation | ↗ la réponse immunitaire innée et adaptative | | (Pathak, 2017) |
| Molybdène (Mo) | Supplémentation | ↙charge parasitaire, ↙ l'excrétion d'œufs, ↗ réponse immunitaire | *H.contortus, T.vitrinus, T.colubriformis* | (Pathak, 2017) |
| Vitamine D3 | Supplémentation | ↗ réponse immunitaire Th2 (↗ lymphocyte circulant et éosinophile activés) | | (Pathak, 2017) |
| Vitamine A | Carence | Retarde l'expulsion des vers adulte, ↗ l'excrétion d'œufs, ↗ la fécondité des vers | | (Pathak, 2017) |
| Vitamine B12 | Carence | Retarde l'expulsion des vers adulte, ↗ l'excrétion d'œufs, ↗ la fécondité des vers | | (Pathak, 2017; Vellema et al., 1996) |
| Vitamine E | Supplémentation | ↙ charge parasitaire, l'excrétion d'œufs, l'anémie | *H.contortus* | (De Wolf et al., 2014) |

**Tableau 6. Principales sources et fonction biologique de 13 vitamines chez le ruminant (INRA, 2018)**

| Noms des vitamines | | Fonctions biologiques | Principales sources naturelles pour les ruminants |
|---|---|---|---|
| A | Rétinol | Vision, immunité, organogenèse et différentiation tissulaire, intégrité cellulaire des épithélia, reproduction | Synthèse à partir des carotènes par les entérocytes |
| | Carotènes | Vision, reproduction, pro-vitamine A | Aliments, principalement les fourrages verts |
| $B_1$ | Thiamine | Métabolisme des sucres et des acides aminés ramifiés | Aliments, synthèse par les bactéries du rumen |
| $B_2$ | Riboflavine | Métabolisme des sucres et des acides gras, production d'énergie | Aliments, synthèse par les bactéries du rumen |
| $B_3$ (PP) | Niacin e | Métabolisme des sucres et des acides gras | Aliments, synthèse par les bactéries du rumen et par le foie |
| $B_5$ | Acid pantothénique | Métabolisme des acides gras et de l'énergie | Aliments, synthèse par les bactéries du rumen |
| $B_6$ | Pyridoxal / Pyridoxine / Pyridoxamine | Métabolisme des acides aminés et synthèse de l'hème | Aliments, synthèse par les bactéries du rumen |
| $B_8$ | Biotine | Transporteur de bicarbonate active pour la carboxylation de substrats, synthèse de glycogène et d'acides aminés | Aliments, synthèse par les bactéries du rumen |
| $B_9$ | Acide folique | Transporteur d'unités fonctionnelles monocarbonées pour le cycle des groupements méthyls impliqué dans la synthèse des acides nucléiques, des lipides, d'hormones, des protéines, de myéline | Aliments, synthèse par les bactéries du rumen |
| $B_{12}$ | Cobalamines | Activation des folates, oxydation des acides aminés ramifiés, des acides gras à chaine impaire, du propionate | Synthèse par les bactéries du rumen |
| C | Acide ascorbique / Acide deshydro-L-ascorbique | Antioxydant hydrophile majeur, agent réducteur participant comme cofacteur enzymatique dans l'hydroxylation du collagène, la synthèse de norépinéphrine, l'amidation d'hormones peptidiques, le métabolisme de la tyrosine | Aliments, synthèse par le foie |
| $D_2$ $D_3$ | Ergocalciférol / Cholécalciférol | Homéostase phospho-calcique, minéralisation osseuse, contraction musculaire, conduction nerveuse | Foin, photosynthèse au niveau de la peau |
| E | Tocophérols | Antioxydant liposoluble majeur dont le rôle est de protéger les composants de la membrane cellulaire (acides gras polyinsaturés) | Aliments (principalement les fourrages verts et les oléagineux) |
| $K_1/K_2$ | Phylloquinone / Ménaquinone | Coagulation sanguine, régulation du cycle cellulaire | Aliments (principalement les fourrages), synthèse par les bactéries du rumen |

### 3.2. Immunologie nutritionnelle

Le système immunitaire a besoin d'énergie, d'AA et de minéraux. Ces nutriments sont nécessaires à la prolifération des leucocytes, à la production des anticorps et à la grande diversité de molécules impliquées dans la réponse immunitaire. Les molécules impliquées dans la fonction immunitaire représentent moins de 2% de la masse corporelle (Paul and Dey, 2015). Les nutriments proviennent à la fois de l'alimentation, des réserves corporelles et de synthèses de novo. La connaissance de la contribution de ces différents compartiments à la réponse du système immunitaire aux SGI repose sur de nombreuses hypothèses plutôt que sur des preuves expérimentales. Des données expérimentales montrent que certaines carences alimentaires pénalisent les fonctions immunitaires qui régulent l'installation, la fécondité et la survie des SGI chez l'hôte (Houdijk et al., 2012). Il y a cependant de nombreuses incertitudes sur la partition et la priorisation de l'utilisation des nutriments issus de la digestion des aliments. Coop and Kyriazakis, (1999) ont émis l'hypothèse que l'expression de l'immunité serait davantage pénalisée que les fonctions productives, car l'allocation de nutriments déficitaires irait préférentiellement à la croissance et à la reproduction. En conséquence, les réponses maximales de production à la supplémentation seraient obtenues avant l'amélioration des fonctions immunitaires (Coop and Kyriazakis, 1999). A l'inverse, Paul and Dey (2015) indique que, du fait de la relative petite taille du système immunitaire, de sa capacité de prélever des nutriments majeurs dans les tissus corporels, et de la présence de transporteurs de nutriments hautement prioritaires, la fonction immunitaire pourrait disposer de nutriments énergétiques et protéiques à partir de rations variées. Ils précisent que le système immunitaire pourrait être plus dépendant de l'alimentation pour certains minéraux tels le fer, le zinc et le cuivre pour lesquels les besoins pourraient être supérieurs à ceux mobilisés pour la croissance et reproduction. Les différentes hypothèses sur la priorisation des flux de nutriments sont probablement à nuancer avec la prise en compte d'éventuelles différences entre les espèces et les génotypes hôtes. La partition de flux de nutriments entre fonctions de production et d'adaptation pourrait être liée au niveau de production des animaux. Les études qui permettraient de valider ces différentes hypothèses sont tributaires d'avancées méthodologiques.

En compilant et analysant au moyen d'une méta-analyse, à même niveau de protéines, des essais où l'ingestion de matière organique avaient largement variée entre les traitements, Ceï et al (2018) montrent que l'apport d'énergie réduit l'excrétion fécale d'œufs (OPG). Ce résultat témoignerait d'un effet positif de l'apport d'énergie sur la résistance des animaux aux

SGI contrairement aux conclusions de la synthèse de Houdijk et al. (2012) qui, sur la base d'une compilation de la littérature, indique que *la résistance de l'hôte aux nématodes gastro-intestinaux est sensible à la rareté des protéines métabolisables et non à la rareté de l'énergie métabolisable.* Houdijk (2012), souligne cependant que dans certains cas, l'apport d'énergie augmente modérément la résistance aux SGI. Cette apparente divergence entre auteurs pourrait en partie s'expliquer par : i) le faible poids relatif du besoin énergétique de la fonction immunitaire relativement aux besoins des autres fonctions qui pourraient être couverts par des réserves présentes dans différents tissus, ii) la difficulté expérimentale de découpler les effets des nutriments énergétiques de ceux des AA du fait des interactions entre métabolisme énergétique et protéique et de la physiologie digestive des ruminants. Concernant ce second point, Houdijk (2012) émet l'hypothèse d'une évolution du système immunitaire qui s'appuierait sur les protéines, ou AA spécifiques, comme source d'énergie. Il indique que diverses cellules immunitaires utilisent de préférence l'alanine et la glutamine comme source d'énergie plutôt que le glucose, et que la glutamine est largement métabolisée dans de nombreuses cellules immunitaires dont les thymocytes, les lymphocytes, les neutrophiles et les macrophages. L'alanine et la glutamine ne sont pas des AA essentiels, ils peuvent être synthétisés par le propre métabolisme de l'animal hôte. La supplémentation énergétique contribue aussi à la synthèse des AA et des protéines microbiennes qui dans une moindre mesure que les protéines alimentaires by-pass, contribuent aux besoins en AA du système immunitaire. «*Le système immunitaire aurait évolué en s'appuyant sur les réserves de protéines de l'organisme plutôt que de dépendre des ressources externes rares* ». Ainsi, en cas de pénurie alimentaire les animaux hôtes auraient la capacité de mobiliser une grande partie des protéines corporelles. En conclusion, l'hypothèse de travail avancée par Houdijk (2012) est une adaptation évolutive qui aurait conduit à une utilisation préférentielle des AA non essentiels comme source d'énergie pour le système immunitaire.

Les ressources protéiques alimentaires se différencient par leur vitesse de dégradation dans le rumen et leur profil d'acides aminés. En conséquence, le profil d'AA absorbés dans l'intestin sera la somme pondérée du profil d'AA microbiens (digestible à plus de 95%) et celui de la fraction digestible des AA alimentaires by-pass dont la digestibilité varie de de 50 à 95% (Houdijk, 2012).

La composition en acides aminées des aliments est très variable. Toutefois, la résistance de l'hôte aux SGI a été mise en évidence avec une grande diversité de sources de protéines alimentaires quand d'importantes quantités sont consommées alors qu'un effet de l'aliment

apparait quand la consommation est plus faible (Houdijk, 2012). La nature des protéines, notamment leur composition en AA, est donc un facteur de variation de la résistance aux SGI. Le profil des AA d'origine alimentaire est cependant variable. Ainsi, des matières premières tels que le soja, la caséine, la farine de poisson ont des effets positifs sur la résistance aux SGI alors que différentes graines de légumineuses donnent des résultats variables. Les stratégies d'alimentation protéiques les plus efficientes seraient donc celles qui augmenteraient l'apport d'AA alimentaires au détriment des AA d'origine microbienne.

Le profil des AA d'origine microbienne est équilibré pour satisfaire les besoins de production (croissance, lait) mais ne serait pas adapté pour répondre aux besoins de synthèse des protéines mobilisées par la fonction immunitaire. La couverture des besoins du système immunitaire en AA dépend principalement de certaines protéines d'origine alimentaire. Les AA impliqués dans la synthèse des protéines spécifiques à la fonction immunitaire est indiquée le Tableau 7 (Li et al., 2007).

La contribution des AA soufrés (méthionine, cystéine) dans la résistance des SGI a été mise en évidence sur différents modèles animaux. Cependant, globalement, la connaissance précise du rôle des différents AA reste à construire.

## Tableau 7. Fonction des acides aminés dans la réponse imunitaire (Li et al 2017)

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| Amino acid | Products | Major functions |
|---|---|---|
| Amino acids | Proteins | Humoral and cellular immune factors and enzymes |
| Alanine | Directly | Inhibition of apoptosis; stimulation of lymphocyte proliferation; and enhancement of Ab production probably through cellular signalling mechanism |
| Arginine | NO | Signalling molecule; killing of pathogens; regulation of cytokine production; and mediator of autoimmune diseases |
| BCAA | Directly | Regulation of protein synthesis and activation of cytokine and Ab production through cellular mTOR signalling |
| | Glutamine | A major fuel for cells of the immune system; regulation of T-lymphocyte proliferation, protein synthesis, as well as cytokine and Ab production; activation of macrophage function; inhibition of apoptosis |
| Cysteine | Taurine | Antioxidant; regulation of cellular redox state |
| Glutamate | GABA | Neurotransmitter; inhibition of T-cell response and inflammation |
| Glutamine | Glu and Asp | Neurotransmitters; components of the malate shuttle; cell metabolism |
| Glycine | Directly | Calcium influx through a glycine-gated channel in the cell membrane |
| | Serine | One-carbon unit metabolism; ceramide and phosphatidylserine formation |
| | Haem | Haemoproteins (e.g. haemoglobin, myoglobin, catalase, and cytochrome *c*); production of carbon monoxide (CO, a signalling molecule) |
| Histidine | Histamine | Allergic reaction; vasodilator; and central acetylcholine secretion |
| | Urocanic acid | Modulation of the immune response in skin |
| Leucine | HMB | Regulation of immune responses |
| Lysine | Directly | Regulation of NO synthesis; antiviral activity |
| Methionine | Homocysteine | Oxidant; inhibitor of NO synthesis |
| | Betaine | Methylation of homocysteine to methionine; one-carbon unit metabolism |
| | Choline | Synthesis of betaine, acetylcholine and phosphatidylcholine |
| | Cysteine | Glutathione synthesis and production of $H_2S$ (signalling molecule) |
| | DCSAM | Methylation of proteins and DNA; polyamine synthesis; gene expression |
| Phenylalanine | Directly | Regulation of tetrahydrobiopterin (a cofactor for NO synthesis) synthesis |
| | Tyrosine | Synthesis of neutrotransmitters that regulate neuronal function and cell metabolism |
| Proline | $H_2O_2$ | Killing pathogens; intestinal integrity; a signalling molecule; immunity |
| | P5C | Cellular redox state; DNA synthesis; lymphocyte proliferation; ornithine and polyamine formation; gene expression |
| Serine | Glycine | Antioxidant; one-carbon unit metabolism; neurotransmitter |
| | Directly | Inhibition of apoptosis; stimulation of lymphocyte proliferation; and enhancement of Ab production probably through cellular signalling mechanism |
| Taurine | TauCl | Anti-inflammation |
| Threonine | Directly | Synthesis of the mucin protein that is required for maintaining intestinal immune function; inhibition of apoptosis; stimulation of lymphocyte proliferation; and enhancement of Ab production |
| Tryptophan | Serotonin | Neurotransmitter; inhibition of the production of inflammatory cytokines and superoxide |
| | NAS | Inhibitor of tetrahydrobiopterin synthesis; antioxidant; inhibition of the production of inflammatory cytokines and superoxide |
| | Melatonin | Antioxidant; inhibition of the production of inflammatory cytokines and superoxide |
| | ANS | Inhibiting production of proinflammatory T-helper-1 cytokines; preventing autoimmune neuroinflammation; enhancing immunity |
| Tyrosine | Dopamine | Neurotransmitter; regulation of immune response |
| | EPN and NEPN | Neurotransmitters; cell metabolism |
| | Melanin | Antioxidant; inhibition of the production of inflammatory cytokines and superoxide |
| Arg and Met | Polyamines | Gene expression; DNA and protein synthesis; ion channel function; apoptosis; signal transduction; antioxidants; cell function; lymphocyte proliferation and differentiation |
| Arg, Met and Gly | Creatine | Antioxidant; antiviral; antitumour |
| Arg, Pro and Gln | Ornithine | Glutamate, glutamine and polyamine synthesis; mitochondrial integrity |
| Cys, Glu and Gly | Glutathione | Free radical scavenger; antioxidant; cell metabolism (e.g. formation of leukotrienes, mercapturate, glutathionylspermidine, glutathione–NO adduct and glutathionylproteins; signal transduction; gene expression; apoptosis; cellular redox state; immune response |
| Gln, Asp and Gly | Nucleic acids | Coding for genetic information; gene expression; cell cycle and function; protein and uric acid synthesis; lymphocyte proliferation |
| | Uric acid | An antioxidant |
| Gln, Glu and Pro | Citrulline | Antioxidant; arginine synthesis |
| Gln and Trp | NAD(P) | Coenzymes for oxidoreductases; substrate of poly(ADP-ribose) polymerase |
| Lys, Met and Ser | Carnitine | Transport of long-chain fatty acids into mitochondria for oxidation; storage of energy as acetylcarnitine |

ANS, anthranilic acid; BCAA, branched-chain amino acids (isoleucine, leucine and valine); DCSAM, decarboxylated *S*-adenosylmethionine; EPN, epinephrine; GABA, γ-amino-butyrate; HMB, β-hydroxy-β-methylbutyrate; mTOR, the mammalian target of rapamycin; NAS, *N*-acetylserotonin; NEPN, norepinephrine; P5C, pyrroline-5-carboxylate, TauCl, taurine chloramine.

## 4. Problématique du projet

### 4.1. Questions de recherche

***Le parasitisme gastro-intestinal des caprins est encore peu étudié***

Le caprin est l'espèce de petit ruminant d'élevage la plus répandue dans la région intertropicale (FAO, 2012). L'impact sur le parasitisme gastro-intestinal est plus important chez les caprins comparativement aux ovins. Cependant, l'essentiel de la littérature sur l'effet de l'alimentation sur les réponses des petits ruminants au parasitisme gastro-intestinal est consacrée aux ovins (Ceï et al., 2018). En conséquence **les principales connaissances acquises sur les caprins méritent encore d'être confortées** car les réponses animales pourraient être différentes entre caprins et ovins (Hoste et al., 2010). Ainsi, une méta-analyse conduite par Ceï et al., (2018) avec un nombre limité d'essais réalisés avec les caprins indique que l'effet de l'apport de l'énergie et de la protéine sur la réduction de l'excrétion fécale d'œufs serait plus faible chez les caprins comparativement aux ovins. Le caprin a donc été retenu comme modèle d'étude dans ce projet.

### 4.2. Effet de l'apport d'énergie et de protéines

Les réponses animales au parasitisme gastro-intestinal entrainent un surcoût nutritionnel pour couvrir les besoins supplémentaires liées à la réparation des cellules endommagées, au renouvellement de cellules prélevées, à la production et au métabolisme de cellules impliquées dans les réponses immunitaires. Le rôle des macro- et micro-nutriments sont maintenant largement démontrés. Cependant l'effet bénéfique de l'apport d'énergie sur la réponse contre les infestations par les SGI chez les petits ruminants mis en évidence par la méta-analyse de Ceï et al (2018) reste à démontrer par l'expérimentation. Quels sont les mécanismes sous-jacents à cet effet bénéfique, notamment sur l'anémie induite par *H. contortus* ? Par ailleurs, la littérature illustre une réponse variable des petits ruminants à la surnutrition protéique. Cette variabilité pourrait être liée au profil des AA absorbés dans l'intestin grêle, résultat de la combinaison d'un profil d'AA d'origine microbienne relativement stable et celui du profil d'AA d'origine alimentaire très variable. Ces deux pools d'AA d'origines différentes ont-ils des effets contrastés sur la réponse animale aux infestations par les SGI ?

### 4.3. Hypothèses de travail

Le travail conduit dans le cadre de ce projet s'articule autour de deux hypothèses principales :

- La « surnutrition » énergétique et protéique contribuent à renforcer la résilience et/ou la résistance des caprins au parasitisme gastro-intestinal.
- L'impact de la « surnutrition » protéique sur la résilience et résistance des caprins au parasitisme gastro-intestinal pourrait être affecté par le profil en acides aminés.

### 4.4. Objectifs du projet

Le présent projet de recherche vise à répondre à 3 principaux objectifs :

- Evaluer l'impact du niveau d'apport d'énergie et de protéines sur les réponses productives et immunitaires de chevreaux aux SGI
- Evaluer l'impact de la source de protéines (profil en acides aminés) sur la résilience et résistance des animaux
- Evaluer la partition des acides aminés (AA) entre la fonction de production et la fonction immunitaire chez les chevreaux.

### 4.5. Démarche de recherche

Ce projet a été construit autour de 4 protocoles qui font l'objet de 4 publications ou projets de publications. La démarche de recherche est résumée par le schéma ci-dessous (Figure 7.).

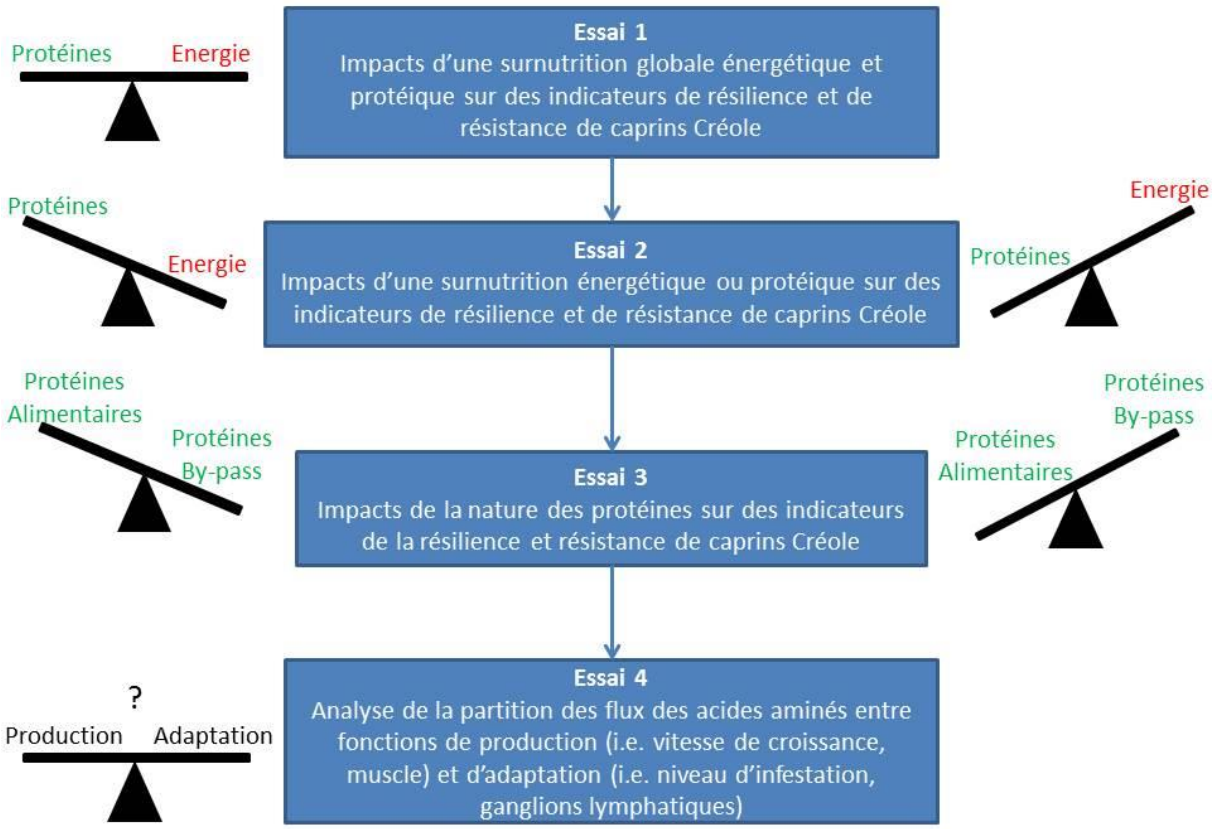


**Figure 7. Schéma conceptuel de la démarche du projet de thèse**

# RÉSULTATS

## **5.1.** ARTICLE 1: THE NUTRITIONAL STATUS AFFECTS THE COMPLETE BLOOD COUNT OF GOATS EXPERIMENTALLY INFECTED WITH *HAEMONCHUS CONTORTUS*

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RESEARCH ARTICLE Open Access

# The nutritional status affects the complete blood count of goats experimentally infected with *Haemonchus contortus*

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## Abstract

**Background:** Gastrointestinal nematode (GIN) remains the most important pathogenic constraint of small ruminant production worldwide. The improvement of the host immune response against GIN though breeding for improved animal resistance, vaccination and nutritional supplementation appear as very promising methods. The objective of this study was to investigate the effect of four nutritional status differing in protein and energy levels (Hay: 5.1 MJ/Kg of dry matter (DM) and 7.6% of crude protein (CP), Ban: 8.3 MJ/Kg of DM and 7.5% of CP, Soy: 7.6 MJ/Kg of DM and 17.3% of CP, BS: 12.7 MJ/Kg of DM and 7.4% of CP) on the haematological disturbances due to *Haemonchus contortus* infection in Creole kid goats.

**Results:** No significant effect of the nutritional status was observed for faecal egg count (FEC) but the experimental infection induced haematological disturbances whose intensity and lengthening were dependent on the nutritional status. A transient marked regenerative macrocytic hypochromic anaemia as revealed by a decrease of packed cell volume (PCV), red blood cells (RBC) and hemoglobin and an increase of reticulocytes was observed in all infected groups except Hay. In this latter, the anaemia settled until the end of the experiment. Furthermore, *H. contortus* induced a thrombocytopenia significantly more pronounced in the group under the lowest nutritional status in term of protein (Hay and Ban). A principal component analysis revealed that the variables that discriminated the nutritional status were the average daily gain (ADG) and the PCV, considered as measures of the level of resilience to *H. contortus* infection. Moreover, the variables that discriminated infected and non-infected animals were mostly related to the biology of RBC (i.e. size and hemoglobin content) and they were correlated with FEC.

**Conclusions:** The severity and the lengthening of the regenerative anaemia and the thrombocytopenia induced by *H. contortus* have been affected by the nutritional status. The protein enriched diets induced resilience to the infection rather than resistance. This suggests that resilience is associated with an improved regenerative capacity of the bone marrow. However, this needs to be further investigated to understand the relationships between resistance, resilience and dietary supplementation.

**Keywords:** Blood cells, Gastrointestinal nematode, Goats, Nutrition

## Background

Gastrointestinal nematode (GIN) infection remains the most important pathogenic constraint of small ruminant production worldwide. The use of chemical drugs as the unique control method is compromised due to the widespread development of anthelmintic resistant GIN populations throughout the world [1, 2]. In addition, the negative possible environmental impacts of anthelmintic residues and the growing consumer demand for chemical-free animal products increase the need for alternative and/or complementary control strategies [3]. The integrated management of GIN infection aims to better control the GIN populations in order to reach a favourable equilibrium for animal production between host and parasites. Thus, numerous axes of research have been developed to control the parasite population both during the host and the free living stages. The improvement of the host immune response against GIN

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though breeding for improved animal resistance, vaccination and nutritional supplementation appear as very promising methods [4, 5].

Nutritional management of small ruminants has long been considered as a tool for the control of GIN infections [6, 7]. It has been suggested that the nutritional status and the capacity of the host to mount an efficient immune response against invading pathogens are closely associated [8, 9]. Indeed, mounting an immune response is both expensive, in terms of proteins, and calories, because of the metabolic requirement of immune cells, the synthesis of proteinaceous immune mediators and the repairing of damaged tissue [10]. Minerals, trace elements and vitamins are also required for the development of immunity [11, 12]. However, numerous studies have shown that the level of nutrition of the host improve either the resilience or the resistance to GIN infections [13, 14]. The resistance is considered as the capacity of the host to develop an efficient protective response and limit the level of parasitism and the resilience as the capacity to limit the pathophysiological consequences of the infection and maintain the level of production. The expression of resilience or resistance would depend on the host genotype, the physiological stage and the quality of the diet mainly in term of protein [15, 16].

Studies that investigate the impact of the nutritional status of infected small ruminants on the interaction between the GIN parasitism and the physiological disturbances are lacking. Therefore the objective of this study was to investigate the effect of the nutritional status on the haematological disturbances due to *Haemonchus contortus* infection in Creole kid goats.

## Methods

### Animals, management and experimental design

This experiment was conducted at the Institut National de la Recherche Agronomique Animal Production Unit (INRA-PTEA, Guadeloupe, French West Indies) experimental facilities (16°N16' latitude, 61°W30' longitude). The Creole goat kids ($n$ = 60, 18.88 ± 3.54 kg body weight (BW); 7 months old) had experienced GIN infection at pasture before been randomly placed indoors in four collective pens ($n$ = 15 kids/pen) corresponding to the experimental groups, 1 month before the experimental infection. The animals were drenched with moxidectine (Cydectine®, Fort Dodge Veterinaria S.A., Tours, France, 300 μg/kg BW) and toltrazuril (Baycox Ovis, Bayer HealthCare, Loos, France, 20 mg/kg BW) and housed under worm-free conditions. During this period, nematode faecal egg counts (FEC) remained at zero. Each group was placed under one of 4 distinct dietary status: Hay (Hay ad libitum non supplemented), Ban (Hay ad libitum + 1250 g of Fresh unripe banana/kids), Soy (Hay +250 g Soybean Meal/kids) and BS (Hay ad libitum + 125 g of Soybean Meal +625 g of Fresh unripe banana/kids). The composition and nutritional values of the diets is shown in Table 1. The supplement, fresh banana (cut into thin slices each day) and/or soybean meal were distributed first and individually with the help of yoke traps during the time of diet consumption.. Thereafter, the hay was distributed ad libitum and animals have free-choice access to fresh water. Feeding stalls were long enough to avoid competition for hay between the kids. The offered hay was adjusted to the groups BW (120% of the maximum intake capacity). After a 1 month period of adaptation to the collective pens and the diet conditions, a total of 10 kids/pen in each group were experimentally infected with a single oral dose of 10,000 *H. contortus* third-stage infective larvae (L3) and 5 kids/pen remain non-infected (infected and non-infected groups on the same diet remained in the same collective pen). The L3 were obtained 35 days before challenge from coproculture of monospecifically infected donor Creole goats with isolates previously obtained from Creole goats reared on pasture in different farms in Guadeloupe [17].

### Growth measurements

The animals were weighed weekly from the day of infection until the end of the experiment to adjust the offered quantities at 120% of the maximum intake capacity according to BW changes and to measure the individual growth rates.

### Faecal egg count

Approximately 10 g of faeces were weekly collected during experimental infection directly from the rectum of each animal to determine the FEC. The faeces were kept

**Table 1** Composition and nutritional values of diets

| | Nutritional conditions | | | |
|---|---|---|---|---|
| | Hay | Ban | Soy | BS |
| Ingredients (g/Kg DM) | | | | |
| Hay | 1000 | 750 | 780 | 762.5 |
| Fresh banana | 0 | 250 | 0 | 125 |
| Soybean meal | 0 | 0 | 220 | 112.5 |
| Chemical composition (%) | | | | |
| OM[a] | 88.2 | 92.9 | 94.2 | 94.7 |
| CP[b] | 7.6 | 8.3 | 17.3 | 12.7 |
| NDF[c] | 65.9 | 50.8 | 46.3 | 45.0 |
| ADF[d] | 32.2 | 25.8 | 23.2 | 21.8 |
| ADL[e] | 4.6 | 3.0 | 2.2 | 2.7 |
| ME[f] (MJ/Kg DM) | 5.1 | 7.5 | 7.6 | 7.4 |

[a]*OM* Organic Matter, [b]*CP* Crude protein, [c]*NDF* Neutral Detergent Fiber, [d]*ADF* Acid Detergent Fiber, [e]*ADL* Acid Detergent Lignin, [f]*ME* Metabolizable Energy

in plastic tubes to avoid contamination and immediately transported from the experimental facility 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 [18].

### Blood cell counts

During the experimental infection, blood samples were individually collected once a week by jugular venipuncture on each animal by using disposable syringes and 20-Ga needles. A 4 mL portion of each blood sample was placed in commercial anticoagulant tubes (ethylenediamine tetraacetic acid $K_3$, EDTA tubes; Becton Dickinson, Plymouth, UK). Blood samples previously placed in EDTA coated tubes were analyzed for a standard haematological profil using a MS4-e (Melet Schloesing Pharmaceuticals s.a., Rue du Collège 90 CH-2300, La Chaux de Fonds, Suisse). The number of circulating eosinophils was determined according to the method of Dawkins et al. [19] with a Malassez cell counter.

### Calculation and statistical analysis

All the animal variables were analyzed by a linear mixed model 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) and the other haematological data were square-root transformed, to normalise residual variances. The model included fixed effects of the days post-infection (T), the dietary status (D), the infection status (I) and the significant interaction between D and I and T as defined below:

$$y_{ijklm} = \mu + \mathrm{D}i + \mathrm{I}j + \mathrm{T}_k + (\mathrm{D} \times \mathrm{I} \times \mathrm{T})_{ijk} + a_{ijl} + +\varepsilon_{jklm}.$$

where $y$ is the observed values; $\mu$ the overall mean; $D_i$ the fixed effect of the i$^{th}$ dietary condition ($i$ = 1 to 4), $I_j$ the fixed effect of the j$^{th}$ infection status (infected vs non-infected), $T_k$ the fixed effect of the k$^{th}$ day post-infection (k = 0 to 49), $(\mathrm{D} \times \mathrm{I} \times \mathrm{T})_{ijk}$ the interaction of the dietary condition, the infection status and the days post-infection, $a_{ijl}$ is the random effect associated with the l$^{th}$ animal in dietary condition $i$ and infection status $j$; and $_{ijklm}$ the random error. All the interactions were initially tested for all variables and only the $(\mathrm{D} \times \mathrm{I} \times \mathrm{T})_{ijk}$ was statistically significant ($P < 0.05$) 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 except growth rate (Average Daily Gain, ADG). The ADG of the animals were estimated by adjusting the weight curve with a linear model. Significance was declared at ≤5% of probability. A principal component analysis (PCA) was performed using the FactoMineR package [20] in R version 3.3.2 (R Cor Team, 2016). The PCA allows to explore the relationships between all the variables (zootechnical, parasitological and haematological) and to describe similarities and differences between animals and associations between variables, to better defined the nutritional and the infection status respectively. The PCA was done with the 13 variables (2 parasitological and zootechnical variables and 11 haematological variables) as active variables and the animals as individuals, ignoring the infection status (I) and the dietary conditions (D). The interaction D × I was included in the PCA analysis as illustrative variable to obtain their coordinates on the different principal components. The two first principal components were then used to plot each animal individually from 28 to 49 days post-infection (d.p.i). corresponding to the period of eggs excretion in the faeces.

## Results

### Parasitological and zootechnical parameters

In the experimentally infected animals, the FEC remained at zero until 28 d.p.i. (Fig. 1). Thereafter FEC increased significantly whatever the dietary status until 49 d.p.i. No significant effect of the dietary status was observed for FEC ($P > 0.05$). No faecal egg excretion was observed in the non-infected animals during the study (data not shown).

A significant interaction was observed between the dietary and the infection status for the ADG ($P < 0.001$, Fig. 2). The ADG was significantly higher in the Soy and the BS groups whatever the infection status ($P < 0.01$). No significant difference was observed between the non-supplemented groups (Hay) whatever the infection status and the non-infected kids of the Ban group ($P > 0.05$). A significant negative effect of the infection status was observed only in the Soy and the Ban groups (−12% and −76% between non-infected and infected kids respectively, $P < 0.05$). The ADG of infected kids was significantly higher in the Hay group compared with the Ban group (48.5 vs. 8.05 g/day respectively, $P < 0.01$). No effect of the infection status was observed in the Hay and the BS groups ($P > 0.05$).

### Haematological parameters

Significant interactions were observed between the dietary and the infection status and the time (d.p.i.) for all the haematological parameters ($P < 0.01$, Fig. 3). A higher variability of the measured parameters was observed in the infected animals. The packed cell volume (PCV), the red blood cells concentration (RBC), the blood hemoglobin concentration, the mean corpuscular hemoglobin concentration (MCHC) and the platelets

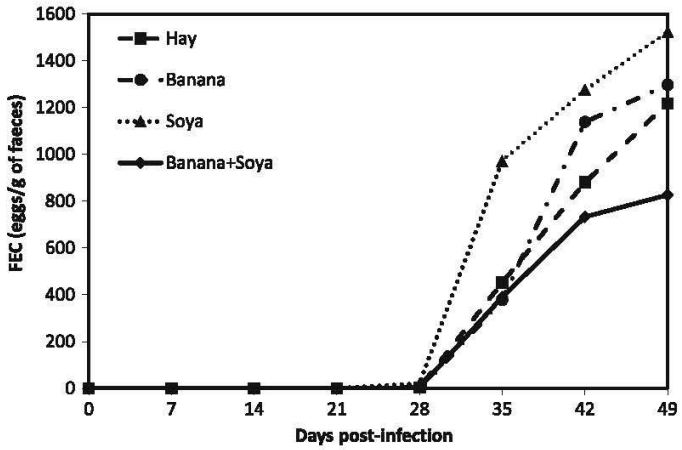


**Fig. 1** Least square means of Faecal egg counts (FEC) according to the nutritional status: Ban: Banana (•, Infected and gray circle, Non-Infected); Hay (■, Infected and gray square, Non-Infected); Soy: Soybean Meal (▲, Infected and gray triangle, Non-Infected); BS: Soybean Meal + Banana (♦, Infected and gray diamond, Non-Infected)

concentration significantly decreased in the infected animals but not in the non-infected ones. In the infected animals, at 21 d.p.i. the Hay group reached the lowest values for these haematological parameters except MCHC and it remained significantly lower than the Ban, Soy and BS groups ($P < 0.01$, Fig. 3). The mean corpuscular volume (MCV) increased significantly ($P < 0.01$) in the infected animals whatever the dietary status. No interaction between time and the dietary status was observed for MCV in the non-infected animals ($P > 0.05$).

### Circulating immune cells

The results for circulating basophils were negligible whatever the dietary or the infection status (data not shown). A significant interaction between the dietary and the infection status and the time was observed for the circulating immune cells except for circulating monocytes ($P < 0.01$, Fig. 4). Circulating lymphocytes and neutrophils decreased slightly but significantly in infected animals ($P < 0.05$). No variation of the circulating monocytes was observed during the course of the experiment whatever the dietary and the infection status ($P > 0.05$). The circulating eosinophils were significantly higher in the infected animals from 14 to 35 d.p.i. ($P < 0.001$). At 35 d.p.i. the circulating eosinophils decreased to the level of non-infected animals in all dietary status except for Hay.

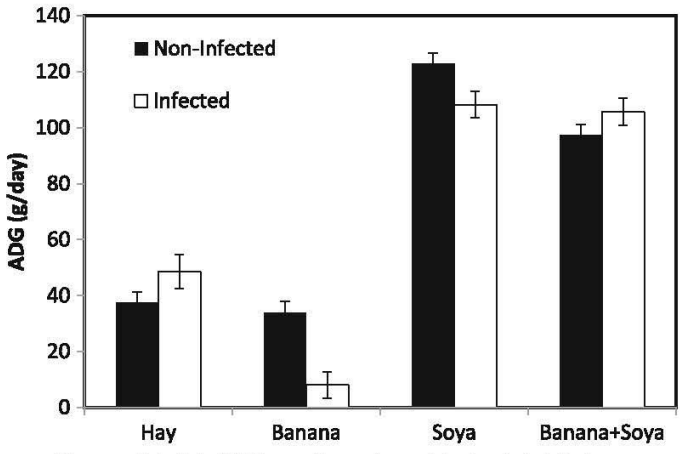


**Fig. 2** Means and standard errors of Average Daily Gain (ADG) according to the nutritional and the infection status

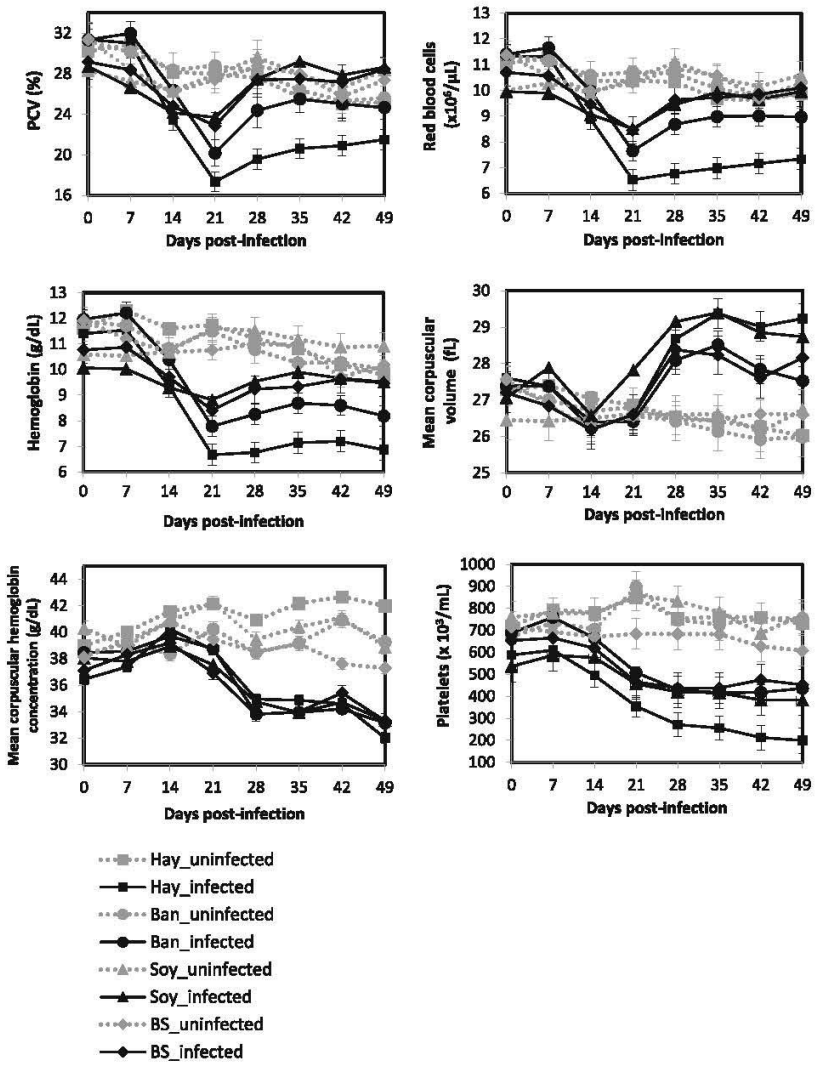


**Fig. 3** Least square means of haematological parameters according to the nutritional and the infection status: Ban: Banana (•, Infected and gray circle, Non-Infected); Hay (■, Infected and gray square, Non-Infected); Soy: Soybean Meal (▲, Infected and gray triangle, Non-Infected); BS: Soybean Meal + Banana (♦, Infected and gray diamond, Non-Infected)

### Principal component analysis of the haemotological, parasitological and zootechnical parameters

The first three principal components of the PCA explained 70.3% of the total variation (34.1%, 19.5% and 16.6% respectively, Table 2). The Fig. 5a shows the contribution of each parameter to the first and the second principal component. The first principal component seemed to contrast FEC, MCV with Platelets, MCH and MCHC. The second principal component seemed to be mainly defined by the contrast between PCV, RBC ADG, and monocytes, lymphocytes and eosinophils. Each animal at each time point, from 28 to 49 d.p.i. was projected on the scatter plot using the two first principal components (Fig. 5b). The infection status (infected and non-infected) was mainly described by the first principal component and the dietary status (Hay, Ban, Soy and BS) by the second principal component.

## Discussion

The nutritional status is closely associated with the intensity of the pathological impact of GIN infection in small ruminants. It has been shown that an improved nutritional status can reduce significantly production losses due to GIN infection by reducing both morbidity

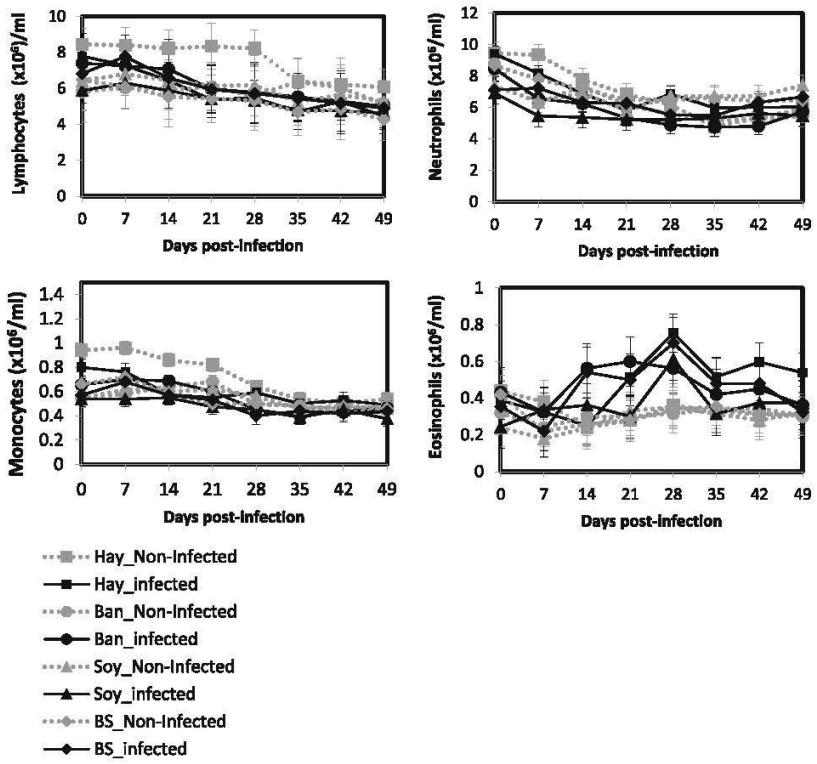


**Fig. 4** Least square means of blood immune cells according to the nutritional and the infection status: Ban: Banana (•, Infected and gray circle, Non-Infected); Hay (■, Infected and gray square, Non-Infected); Soy: Soybean Meal (▲, Infected and gray triangle, Non-Infected); BS: Soybean Meal + Banana (♦, Infected and gray diamond, Non-Infected)

and mortality [21, 22]. In *H. contortus* infection, morbidity and mortality are mainly caused by the haematophagous activity of both larval and adult stages of the parasite whose consequence is an irreversible loss of protein. The objective of this study was to investigate the effect of the nutritional status on the haematological disturbances due to *H. contortus* infection in Creole kid goats. No significant effect of the nutritional status was observed for FEC. In accordance with a previous study, a single bolus oral experimental infection of Creole kid goats that previously experienced natural GIN infection at pasture induce a low level of parasitism measured through the FEC and the lengthening of the prepatent period [17]. Nonetheless, we showed a significant interaction between the nutritional and the infection status on the growth rate. The animal placed under the lowest nutritional status in term of protein (i.e. Hay and Ban) showed the lowest growth rate and the experimental infection reduced the growth rate only for animals placed in the Ban and the Soy groups. Furthermore, the experimental infection induced haematological disturbances whose intensity and lengthening were dependent on the

**Table 2** Estimate of eigenvalues (λj), percentage of variance (λ%), and cumulative variance and eigenvectors associated to the three first principal components of the animal responses during an experimental *H. contortus* infection (from 28 to 49 days post-infection)

| F[a] | λj | λ(%) | Cumulative variance (%) | Associated eigenvectors[b] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ADG[b] | FEC[c] | PCV[d] | RBC[e] | Hgb[f] | MCV[g] | MCH[h] | MCHC[i] | Plat[j] | Lym[k] | Mon[l] | Neut[m] | Eos[n] |
| 1 | 4.78 | 34.14 | 34.14 | 0.26 | −0.36 | 0.48 | 0.66 | 0.84 | −0.50 | 0.63 | 0.71 | 0.80 | 0.67 | 0.55 | 0.50 | 0.24 |
| 2 | 2.73 | 19.49 | 53.63 | 0.59 | 0.03 | 0.76 | 0.67 | 0.52 | 0.25 | −0.13 | −0.23 | 0.13 | −0.48 | −0.56 | −0.29 | −0.31 |
| 3 | 2.33 | 16.63 | 70.27 | 0.31 | 0.31 | 0.37 | 0.18 | −0.02 | 0.55 | −0.44 | −0.61 | −0.44 | 0.54 | 0.42 | 0.53 | 0.16 |

[a]*F* Functions (Principal components), [b]*ADG* Average Daily Gain, [c]*FEC* Feacal Egg counts, [d]*PCV* Packed Cell Volume, [e]*RBC* Red Blood Cells, [f]*Hgb* Hemoglobin, [g]*MCV* Mean Corpuscular Volume, [h]*MCH* Mean Corpuscular Hemoglobin, [i]*MCHC* Mean Corpuscular Hemoglobin Concentration, [j]*Plat* Platelets, [k]*Lym* Lymphocytes, [l]*Mon* Monocytes, [m]*Neut* Neutrophiles, [n]*Eos* Eosinophils

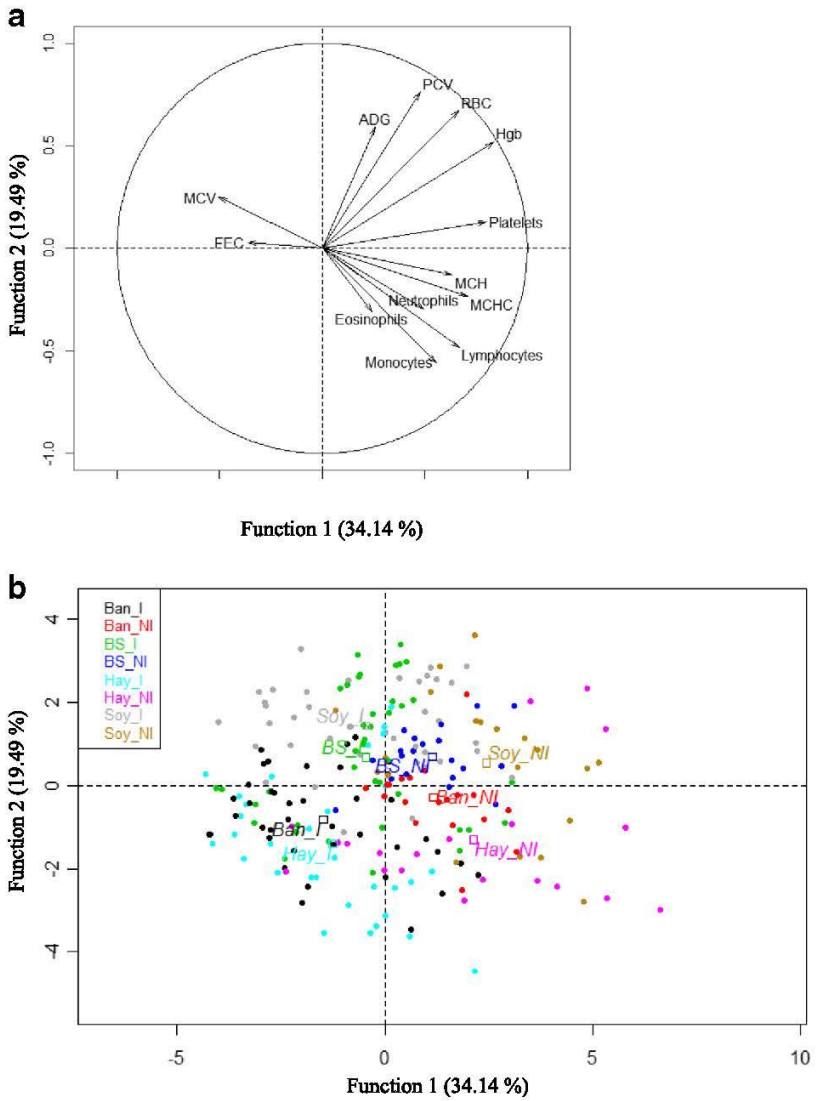


**Fig. 5** Principal component analysis of the haematological, parasitological and zootechnical parameters. **a** Mapping of the parameters and (**b**) Representation of the animals according to the dietary (Ban: Banana; Hay; Soy: Soybean Meal; BS: Soybean Meal + Banana) and the infection (I: experimentally infected with *Haemonchus contortus*; NI: Non-infected) status from 28 until 49 days post-infection on two-dimensional scatter plot. FEC: Faecal egg counts; MCV: Mean Cell Volume; ADG: Average Daily Gain; PCV: Packed Cell Volume; RBC: Red Blood Cells; Hgb: Hemoglobin; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration

nutritional status. A transient marked anaemia as revealed by a decrease of PCV, RBC and hemoglobin concentrations was observed in all infected groups except Hay. In this latter, the anaemia settled until the end of the experiment. Interestingly, the concentration of hemoglobin (MCHC) did not increase proportionally with the increase in RBC size (MCV). These RBC with a larger volume and a lower hemoglobin concentration were probably reticulocytes (i.e. immatures RBC) as previously shown [23]. These results were in accordance with previous studies conducted in sheep, characterizing the type of anaemia induced by *H. contortus* as regenerative macrocytic hypochromic anaemia [24, 25]. Indeed, the pathogenesis of regenerative anaemia includes external hemorrhage which is chronic in the case of *H. contortus* infection. Furthermore, in the present experiment

the number of platelets decreased in all infected animals. This thrombocytopenia was significantly more pronounced in the Hay group, as for PCV, RBC and hemoglobin. Similarly, it has been shown in *H. contortus* infected sheep that the number of platelets decreased progressively during the course of *H. contortus* infection [23]. To our knowledge, besides this study in sheep and ours in goats, this result has never been described in the literature. However, a platelet aggregation and adhesion inhibitor from adults *H. contortus* has been identified and characterized in vitro [26]. It must be emphasized that the host blood taken from the abomasal mucosa is the main nutrient source of the parasitic stage of *H. contortus*. Altogether, these results strongly suggested that *H. contortus* has developed a broad-spectrum strategy to manipulate the host's hemostatic system, which would target especially the blood platelets.

With the exception of eosinophils, the blood leucocytes counts were not affected by the infection and the nutritional status. Indeed, many studies showed significant correlations between resistance/susceptibility to GIN infection and the magnitude of the peripheral blood eosinophil [27]. Even though this relationship has not been observed in all studies, it is largely admitted that peripheral blood eosinophil plays a key role in the protective response to GIN [28–30]. In contrast with a previous study in Creole goat [31], blood eosinophil counts were not affected by the nutritional status. In the present study the nutritional status were not as contrasted as it was previously. On the other hand, it has been shown that peripheral blood lymphocytes are also involved in the protective response to GIN. A significant negative phenotypic correlation has been found between blood lymphocyte counts and *H. contortus* fecundity [32]. In contrast, in another sheep breed, lymphopenia was observed in *H. contortus* infected lambs [33]. However, the close association between the resistance to GIN infection and the host immune response has been demonstrated in a study showing that depletion of CD4+ T lymphocytes significantly increased the parasitic load in a resistant sheep breed [28]. In Creole goats experimentally infected, we have showed that the level of circulating activated CD4+ and CD8+ T lymphocytes were higher in susceptible animals compared with resistant [34]. Altogether these results obtained in different sheep breeds and goats, with different level of parasitism confirmed the effective role of lymphocytes in the host response against GIN, but also emphasize the need to better investigate this relationship.

The comparison between the Hay and the Ban groups showed that infected animals in the Hay groups prioritize growth at the expense of a marked pathological impact while the reverse was observed in the Ban group. These results were partly in keeping with previous studies suggesting that an improved nutritional status (here Soy and BS compared to Hay and Ban) induced resilience rather than resistance to the experimental *H. contortus* infection (i.e. reduced pathological impact for the same parasitic load) [25, 35, 36]. Interestingly, the PCA revealed that in our study the variables that discriminated the nutritional status were ADG and PCV, considered as measures of the level of resilience to *H. contortus* infection, and eosinophils. Moreover, the variables that discriminated infected and non-infected animals at the cellular level were mostly related to the biology of red blood cells (i.e. size and hemoglobin content) and they were correlated with FEC. The association of eosinophils with resilience was not surprising in our animal model since we had previously shown that, in contrast with numerous studies in sheep, blood eosinophil counts were associated with the level of infection rather than the protective response in goats [34].

Otherwise, other studies including one of ours, have also reported resistance to GIN (i.e. reduced pathological impact and parasitic load) induced by an improved nutritional status [31, 37, 38]. Discrepancy between the different studies could be attributed to the animals physiological stage which would influenced the trade-offs between the immune response against invading pathogens and others physiological functions [39]. The quality of the metabolizable protein arising from the rumen (microbial protein vs by-pass protein), could also be an explanation since the amino acid composition of immune proteins is more compatible with that of by-pass proteins [16]. Further research is needed to better understand the nutrition × parasitism interaction in small ruminant for a better fine tuning of the technical recommendations to breeders.

## Conclusions

The results showed that infection with *H. contortus* induced a regenerative anaemia and a thrombocytopenia. The severity and lengthening of these pathological disturbances have been affected by the nutritional status, the protein enriched diets induced resilience to the infection rather than resistance. This suggests that resilience is associated with an improved regenerative capacity of the bone marrow. However, further investigation to understand the relationships between resistance, resilience and dietary supplementation.

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**Availability of data and materials**
All data supporting the results of this study are included within this article.

**Authors' contributions**
JCB, HA and SC conceived and designed the experiments, CJ and RA and the Gardel team collected samples for the haematological and parasitological analysis. DF and YF performed the haematological and parasitological analysis. JCB and SC performed the statistical analysis and wrote the paper. All authors read and approved the final manuscript.

**Competing interest**
The authors declare that they have no competing interests.

**Ethics approval and consent to participate**
All animal care, handling techniques, procedures as well as license for experimental infection and blood sampling were approved by the current law on animal experimentation and ethics (HC-69-2014-1 from the Animal Care and Use Committee of French West Indies and Guyana), 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.

**Consent for publication**
Not applicable.

## Publisher's Note



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## **5.2.** ARTICLE 2 : EFFECT OF ENERGY AND PROTEIN SUPPLEMENTATION ON RESILIENCE AND/OR RESISTANCE OF CREOLE KIDS GOATS FOLLOWING AN EXPERIMENTAL *HAEMONCHUS CONTORTUS* INFECTION

## Effect of energy and protein supplementation on resilience and/or resistance of Creole kids goats following an experimental *Haemonchus contortus* infection

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## Abstract

The objective of this study was to investigate the effect of energy and protein input on the resilience and resistance of Creole goat kids to an experimental *Haemonchus contortus* infection. Fifty six kids were fed with 4 diets: Hay (Hay *ad libitum*); HB (Hay *ad libitum* + Banana); HS (hay *ad libitum* + soya meal) and HSB (Hay *ad libitum* + Banana+ Soya meal). In each diet group, 8 kids were experimentally infected with 10000 *H. contortus* infective larvae (L3) and 6 kids were kept as non-infected control. From the day of infection until 6 weeks post-infection, samples were collected to estimate individual intake, total tract digestibility, parasitological, haematological and immune parameters. Diets have a significant effect on average daily gain (ADG) but no interaction with the infection was observed. Diets have a significant negative effect on the female worm burden. There seems to be a relationship between the female worm burden and their prolificity. The resilience of kids increased with the input of protein. No effect of energy input was observed on resilience of kids.

**Key words**: *Haemonchus contortus*, goat kids, protein and energy supplementation

## Introduction

For a long time, the main strategy against gastrointestinal parasitism was based on the use of chemical molecules with the objective of eradication. This strategy has shown its limitations because gastrointestinal nematodes (GIN) have developed resistance mechanisms against

almost all the chemical molecules available (Jackson and Coop, 2000; Mahieu et al., 2014; Papadopoulos et al., 2012). Regardless of this agronomic issue, in Europe, agricultural policies advocate a reduction in the use of these molecules due to likely negative impacts on the environment and biodiversity (Beynon, 2012). Moreover, consumers are more and more legitimately reluctant to eat animal products that may contain residues of these chemical molecules.

During the last twenty years, research efforts have focused on three main axis: i) the reduction of the risk of contact between ruminants and GIN mainly by grazing management strategies; ii) the improvement of the host resistance by genetic selection or nutritional strategies and iii) the reduction of the use of chemical anthelmintic by targeted drenching and the use of neutraceutical plant resources. Furthermore, today the objective is not the search for an unique solution but rather integrated control strategies of GIN combining different solutions (Waller, 1999). Gastro intestinal parasitism is often assimilated to a nutritional disease because of its major negative impacts on diet intake and total tract digestibility, the reorientation of nutrient use for repairing of damaged tissue and mounting an immune response rather than for production functions (Hoste et al., 2005). Consequently, the nutritional strategy, which is generally over-nutrition with concentrate in relation to conventional recommendations, aims to provide nutrients that enable the animal to meet the needs of the immune system and compensate for losses caused by the GIN. The nutritional strategy integrates the quantitative and qualitative aspects of macro and micronutrients (Paul and Dey, 2015).With regard to macronutrients, work has focused on protein intake and, secondarily, on energy intake (Houdijk, 2012).

In a meta-analysis Ceï et al (2018) confirm some knowledge and hypotheses concerning the positive impacts of energy and protein on the resilience of sheep and goats to GIN infections. They have shown a significant variability of the response to protein input and a weak

experimental evidence on the effect of energy input. Moreover, this meta-analysis underlined the need for more studies in goats.

Recently, Cériac et al. (2017) showed that protein over-nutrition increased resilience but not resistance of goat kids by reducing anemia through an increased hematological regeneration capacity of the bone marrow. The effect of energy intake was not evaluated in this study since individual intake and digestibility was not performed. In the present study, we monitored individually the animal response to evaluate the respective effect of energy and protein intake on the responses of Creole goat kids to an experimental *Haemonchus contortus* infection.

## Materials and methods

### Animals, diets and experimental design

The trial was carried out in 2017, in the experimental animal facilities of the French National Agronomic Research Institute (INRA) of the West Indies (Guadeloupe, latitude16.16 N, longitude 61.30 W). All animal care, handling techniques, and procedures as well as the license for experimental infection and blood sampling were approved by INRA, according to the certificate of authorization (number A-971-18-02) to experiment on living animals issued by the French Ministry of Agriculture, before the initiation of the research. The trial lasted 4 months. All the kids were raised on pasture and consequently had undergone a natural GIN infection before the experiment. The kids were drenched with Levamisole (Polystrongle ®, Merial, Lyon, France, 7,5mg/kg LW), Ivermectine (Oramec®, Merial, Lyon, France, 0,2 mg/kg LW) and Praziquantel (Cestocur®, Byer santé, Puteaux Fance, 3,75mg/kg LW) before being placed indoors in individual cages for the experiment. Thereafter, the kids had a 7-week adaptation period to the diets. Then, during 3 weeks, measurements were performed on the 56 non-infected Creole male kids. On the basis of the estimated breeding values, half of the kids were resistant to parasitism and the other were susceptible. These animals were

homogeneously distributed in the different experimental groups. Finally, during 6 weeks, these kids were placed into a group of 24 kids used as non-infected controls (NI) and a group of 32 kids infested (I) orally with a single dose of 10000 *H. contortus* stage 3 larvae (L3) obtained by coproculture of faeces provided by donor kids (Bambou et al., 2008).

Four diets were evaluated: 1) Grass hay *ad libitum* (HAY); 2) Grass hay *ad libitum* + 1250g slices green banana (HB); 3) Grass hay *ad libitum* + 250g of soybean meal (HS); 4) Grass hay *ad libitum* + 250g of soybean meal + 1250g slices green banana (HBS). Eight experimental groups were constituted to combine two infection status (i.e. infected vs non-infected) and 4 diets.

The chemical composition of the ingredients of the diets is presented in Table 1.

**Animal samples, diet samples and measurements**

The kids were weighed at the beginning of the experiment and every week until the end of the trial. Average Daily Gain was estimated by regression of live weight over time. Intake (DM, OM, CP, NDF, and ADF), apparent total-tract digestibility (OM, CP) were performed. Individual intake and digestibility were determined from daily weighing of the amounts of diet offered and refused and the feces during 5 consecutive days during 7 periods (pre- and post-infection) over the entire duration of the experiment. Kids were equipped with bags glued at the back for harvesting feces. Daily representative samples of the forage offered and refused and of feces samples were taken, dried at 60°C for 3 days in a ventilated oven to determine the dry matter content and in planning for chemical analyses.

After infection, blood samples of each kid were collected once a week by jugular venipuncture using disposable syringes and 20-Ga needles. A 2.5-mL blood sample was collected in commercial anticoagulant tubes (ethylenediamine tetraacetic acid K3, EDTA tubes; Becton Dickinson, Plymouth, UK). Blood samples were analyzed by an automaton

(Melet Schloesing, MS9-5s, Osny, FRANCE), to measure Pack Cell Volume (PCV), red blood cells rate (RBC), blood platelets, mean corpuscular volume of the red blood cells (MCV), neutrophils, lymphocytes, monocytes and basophils rate as well as circulating eosinophils.

Blood samples collected in plastic tubes (Becton Dickinson, New Jersey, USA) were centrifuged for 5 min. at 5000 rpm at 4°C, and serum was then frozen at −20◦C until analysis. Serum pepsinogen levels were determined using a micro method for routine determination according to (Dor-ny and Vercruysse, 1998). The serum pepsinogen level, measured weekly, is used as an indicator of the mucosal damage caused by *H. contortus* infection. 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.

Fecal egg count (FEC, eggs/g feces), was estimated using a modified McMaster method for rapid determination (Aumont 1997). After the experimental infection, approximately a 10 g fecal sample was collected weekly on each kid directly from the rectum. The feces were kept in plastic tubes to avoid contamination and immediately transported to the laboratory in refrigerated vials for egg counting.

**Chemical analysis**

The dry matter (DM) content of feed proposed, refused and feces excreted were determined by drying at 60°C for 3 days. Diet and feces samples were milled through a 1-mm screen (Reich hammer mill, Haan, Germany) prior to analysis. Organic matter (OM) and N analyses were performed according to AOAC (1990, Methods 923.3 and 992.15, respectively) by ashing at 550°C for 6 h for OM and by the Dumas method for N. Nitrogen analyses of fresh urine samples were performed according to the same method as for the diets. Crude protein (CP) was calculated as N  6.25. Cell wall components (neutral detergent fibre (NDF), acid

detergent fibre (ADF) and ADL) in diet and faeces were determined using a sequential procedure (AOAC, 2006, Methods 200.04 and 973.18, respectively for NDF and ADF and ADL).

**Statistical analysis**

Data were analyzed by a linear mixed model using the PROC MIXED of SAS (Version 9, SAS Inst., Inc., Cary, NC, 1999). FEC and eosinophilia variables were logarithm transformed (ln (FEC + 15), ln (Blood eosinophils + 1) respectively) and the other hematological data were square-root transformed, to normalize residual variances.

The model included fixed effects of the diet (D), the infection status (I), the weeks post-infection (T), the genotype (G) and the interaction between D and I as defined below:

$$y_{ijklm} = \mu + D_i + I_j + T_k + G_l + (D_i + I_j)_{ij} + \varepsilon_{ijkl}$$

where y is the observed values; $\mu$ the overall mean; $D_i$ the fixed effect of the ith dietary condition (i = 1 to 4), $I_j$ the fixed effect of the $j^{th}$ infection status (infected vs non-infected), $T_k$ the fixed effect of the $k^{th}$ week post infection (k = 0 to 6), Gl the fixed effect of the $l^{th}$ genetic status, $(D \times I)^{ij}$ the interaction of the diet and infection status.

These same analyses were carried out taking into account the fixed effects when significant.

## Results

No significant effects of genetic status were observed.

**Intake and digestion**

The mean DM, OM and digestible OM intakes during the trial were significantly higher and lower with HSB and HAY diets respectively ($P < 0.05$, Table 2). Intakes of HS and HSB diets were similar. HB diet had an intermediate value between the extreme diets and was

significantly different to these diets. CP and digestible CP intakes increased according to the hierarchy HB, HAY, HBS and HS diets (Table 2). The differences were significant between the diets with the exception of HAY and HB for the CP intakes ($P < 0.05$). Intake (OM, CP) slightly varied over time probably in relation with forage quality (Fig.1ab). No significant effects of infection status were observed. Similarly, no diet x infection interaction was significant. Total tract digestibility (OM, CP) slightly varied over time but no significant effects of infection status and no significant diet x infection interaction were observed (Fig. 2ab). No significant effects of infection status, no significant diet x infection interaction were observed on digestible OM and CP intakes.

**Parasitological and zootechnical parameters**

The FEC remained at zero until 2 weeks post-infection then increased significantly whatever the dietary status until 6 weeks post-infection (Fig. 3). There was no interaction between the diet and the time post infection. No significant effect of the diet was observed on the FEC and the nematode burden (Fig. 3 and Table 3). In contrast, a significant effect of the diet was observed on the female nematode burden and the prolificacy (Table 3, $P < 0.05$). The female nematode burden was significantly higher in the Hay and the HB diets compared with the HS and the HSB diets ($P < 0.04$). The prolificacy of the female nematode of the HAY and the HB diets was not different, but lower in HAY diet compared with HSB diets, and in the HB diet compared with the HS and HSB one ($P < 0.05$).

There is a significant effect of the diet on the ADG ($P < 0.0001$, Fig. 4). The highest ADG were observed with HBS, the lowest were observed with HAY and HB which were not significantly different. No significant effect of the infection was observed on the ADG whatever the diet.

**Haematological parameters**

With the exception of MCV (mean corpuscular volume), there was a significant interaction between the diet, the time post-infection and the infection for the hematological parameters ($P < 0.05$, Fig.7). Globally, the PCV increased significantly according to the hierarchy HB, HAY, HS and HBS ($P < 0.05$). The PCV was significantly lower in the infected kids compared with the non-infected ones whatever the diet ($P < 0.05$). These differences were observed at weeks 3, 4, 5 and 6 for HB and HAY, weeks 4 and 5 for HS and weeks 4, 5 and 6 for HSB (Fig.5).

Globally, RBC increased with hierarchy HB, HAY, HS and HBS for non-infected and infected kids. The RBC rate was significantly lower in the infected kids compared with the non-infected ones whatever the diet ($P < 0.05$, Fig. 6). These differences were observed at weeks 3, 4, 5 and 6 for HB diet, weeks 5 and 6 for the HS diet, weeks 4, 5 and 6 for the HSB diet and weeks 3 and 4 for the Hay diet. Infected kids under the HS diet and the HSB diet had a significant higher RBC than infected kids under the HB diet ($P < 0.05$). Infected kids under the HSB diet had significantly higher RBC than infected kids under the hay diet ($P<0.05$).

High significant effects of the infection status, the diet and the weeks post infection were observed on the mean corpuscular volume (MCV) ($P<0.0001$, Fig. 7). The MCV increased significantly overtime in infected kids. Kids under the HS diet or the HSB diet had significantly higher MCV rate than kids fed with the HB diet or Hay. The MCV was not significantly different between the HAY and the HB diet and was significantly different between the HS diet and HSB diet ($P < 0.05$).

High significant effects of the infection status ($P < 0.0001$), the diet ($P< 0.002$) and weeks post infection ($P < 0.0001$) were observed on blood platelet and mean platelet volume (Fig. 8). Blood platelet was lower in infected kids compared non infected kids ($P < 0.0001$). Kids

fed with the HB diet had significant higher blood platelet than kids fed with HS diet or HSB diet. The blood platelet rate of infected kids decreases significantly over time ($P < 0.0001$). The mean platelet volume was lower in infected compared with the non-infected kids. The mean platelet volume increase with the hierarchy HB, HAY, HS and HSB diets.

High significant effects of the infection status ($P < 0.0001$), the diet ($P<0.0001$) and weeks post infection ($P < 0.0001$) were observed on blood pepsinogen concentration (Fig. 13). Blood pepsinogen was higher in infected kids compared non infected kids ($P < 0.0001$). Kids fed with the HSB diet had the higher blood pepsinogen concentration. Kids fed with the HAY diet had the lower blood pepsinogen concentration. The blood pepsinogen concentration of infected kids increases significantly over time and then decreases after the 4th day ($P < 0.0001$)

**Circulating immune cells**

There was no interaction between the diets, the weeks post infection and the infection for the circulating immune cells analyzed ($P > 0.05$). The blood eosinophils count was higher in the infected kids under the HAY diet compared to non-infected kids ($P < 0.001$, Fig. 9). Within the infected kids, the lowest blood eosinophils count was observed for the HSB diet and the highest for the Hay diet.

A significant effect of the diet was observed on the blood lymphocytes and monocytes concentration but not for the concentration of basophils ($P < 0.05$, Fig. 10 and Fig. 11, respectively). No significant effect of the infection status was observed on these parameters ($P > 0.05$) except for lymphocytes ($P < 0.01$). A high interaction between the diet and the infection status was found for blood neutrophils ($P<0.0001$, Fig.12). Moreover, no significant effect of the time post infection was observed on blood basophils and on the neutrophils. All the infected kids had a similar neutrophils rate whatever the diet. However, in non-infected

kids the highest neutrophils rate was found for the HS diet ($P < 0.05$). No significant difference for the blood neutrophils was found for kids under the HB, HSB and Hay diets. The blood monocytes were lower in kids which were under the HSB diet compared with the HB, HSB and the Hay diet ($P < 0.01$).

## Discussion

The objective of this study was to evaluate the respective effect of energy and protein intake on the responses of Creole goat kids to an experimental *Haemonchus contortus* infection. We formulated four diets to evaluate: i) the impact of energy input in diet covering half of the growth potential of Creole kids (HAY *vs.* HB diets); ii) the impact of energy input in diets covering the growth potential of Creole kids (HS *vs.* HBS diets) and iii) the impact of protein over-nutrition relatively to Creole kids growth potential (HS diet). Unfortunately, the level of substitution of hay intake by fresh banana in the HB diet has somewhat counteracted this design. We rather compared different nutritional status than contrasted levels of energy and protein intakes. The differences in intake and digestion between diets reported here are classical results due to their chemical composition. No impact of the experimental infection on DMI was observed whatever the diet. Indeed, even if some authors reported a significant decrease of DMI induced by GIN infection this is not a general trend (Ceï et al., 2018; Coop and Kyriazakis, 2001; Dakkak, 1995). Anorexia would be more common with naïve young animals when activating their immune system. During this activating immune system period, DMI decreased from 20 to 50% against less than 5% in sheep with a mature immune system (Bambou et al. 2009; Sykes, 2010). In keeping with our results, decrease of diet digestibility has also been reported, especially with GIN established in the abomasum (Ceï et al., 2018). However these reductions has been associated with very high levels of infection and can be

explained by a hypermotility of the gastro-intestinal tract, which accelerates the transit of digesta and increased protein losses in the gastro-intestinal tract (Bueno et al., 1982).

The diets were formulated on the basis digestible OM intake/$LW^{0.75}$ and digestible CP intake/$LW^{0.75}$ to generate a gradient in energy and protein availability for the metabolic production functions and immune responses of kids. The energy availability would be logically higher with the HB diet compared to HAY in accordance with our expectations. Contrary to our working hypothesis, protein availability was lower with HB diets, compared with HAY diets because fresh bananas were consumed at the expense of hay (55.5 vs 91.5 g/kg DM of crude protein). As a result, HAY and HB diets were not contrasted since no difference of ADG was observed between these diets. The protein level was the first limiting factor of these diets, consequently ADG were the lowest observed in this study and the evaluation of the specific effect of energy input was not possible. Similarly, the HS and the HBS diets were not discriminant in energy because the diet formulated provided amounts of energy and proteins above the growth requirements. A significant effect of the diet was observed on the adult female nematode burden and the prolificacy but not on the FEC. Therefore, our results indicate that on the basis of the FEC and the serum pepsinogen, an indicator of the level of tissue damages, the amount of energy and proteins did not allow the Creole kids to develop resistance against the *H. contortus*. However, for the same FEC, the adult female nematode burden was lower and the prolificacy was higher with the HS and the HSB diets. The highest nutritional status would allow the control of the nematode establishment but not the prolificacy which would be probably modulated according to the worm burden. These results are in contradiction with the studies of Bricarello et al (2005) or Blackburn (1991), which reported that protein supplementation contributes to increase the host resistance (i.e. lower FEC and worm burden). Nonetheless, our results are in accordance with Wallace et al (1996) on lambs suggesting that protein supplementation could improve the

capacity to withstand the pathogenic effect of *H. contortus*. The *H. contortus* infection disturbed the hematological parameters. The PCV as well as the RBC, which reflects the anemia level of the animal, were both significantly lower in infected kids compared with non-infected kids, confirming the anemia classically present with this pathology (Ceï et al.,2018). Kids fed with a higher nutritional status in term of proteins (HS and HSB) had significantly lower anemia than the other kids, illustrating the potential of over nutrition to increase the resilience of infected kids.

Infected kids had higher MCV than non-infected ones as already reported with Cériac et al (2017). The latter authors indicated that the RBC of infected animal was probably reticulocytes characterized by a larger volume associated with a lower hemoglobin concentration, similar to what we found concerning the PCV and RBC parameters. Those reticulocytes, which are the young form of the RBC synthesis by the bone marrow, should be the consequence of the induction of regenerative anemia to cope with the *H. contortus* infection, as in the studies of Khan et al (2012) and Abbott et al (1986). A higher MCV has been observed in kids under the highest protein nutritional status (HS and HSB) whatever the infection status. In the present study, the lowest adult female nematode burden observed in the highest protein nutritional status (HS and HSB) could also explain at least in part the lowest anemia. A similar result has been described by the study of Konwar et al (2015) where they added various levels of protein concentration in the goat feed. They found that an addition of feed containing at least 20% or 24% of digestible crude protein had a positive impact on the MCV after 30 days compared to goat without protein supplementation. A hypothesis is that the proteins could have stimulated the production of erythropoietin as the main action of this molecule is to improve the RBC synthesis and growth. Indeed, Rosenberg et al (1989) showed that a high protein diet could stimulate erythropoietin production in kidney disease in human. Furthermore, Brown and Shapiro (1996) showed that high protein intake improved erythroid

differentiation in very low birth weight infants. The main action of erythropoietin is to increase the survival of erythroid progenitors which then differentiated into reticulocytes (Koury and Bondurant, 1990). The blood circulating immune cells analyzed in the present study were not impacted by the experimental infection of the kids, except for the concentration of lymphocyte. However, those circulating immune cells were affected by the dietary status, except for neutrophils and monocytes. Eosinophils are the only white blood cells described which react to a parasite infection, so an increase of this parameter was expected after experimental infection (Bambou et al., 2013). A higher blood circulating eosinophils concentration has been found in kids consuming the protein diet as well as in the ones which were fed the energy diet. This could be explained by the fact that proteins are essential nutrients which plays a central role in the immune system (Daly et al., 1990). Concerning neutrophils, in the study of Konwar et al (2015), they also observed a decrease of the neutrophils rate in infected kids fed with an enriched protein diet even if it was not significant. Our results indicate that kids under the protein-enriched diet had the lower monocytes rate. Those results are in contradiction with Konwar et al (2015) who found that monocytes percentages were not significantly influenced by a protein supplementation.

## Conclusion

Among the different diets evaluated in this study, none of them allowed to confer a resistance against *H. contortus*. The nutritional status containing protein supplementation allowed the kids to develop resilience against *H. contortus* by an increased RBC synthesis by the bone marrow and a limited adult female nematode establishment. No difference of feed intake or digestibility was observed but ADG, blood eosinophils counts as well as tissue repair were improved with protein supplementation, which confirmed the implication of proteins in the induction of resilience. Even if a protein supplementation seemed to improve the resilience,

an association with energy had a better effect on the resilience, mostly concerning the immune system.

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Table 1. Chemical composition of *Dichantium sp.* Hay, soybean meal and green banana

| | Feeds | | |
|---|---|---|---|
| Items | Hay | Soybean Meal | Banana |
| Dry matter, g/kg | 830.0 | 890.0 | 210.0 |
| Organic matter, g/kg DM | 912.1 | 914.9 | 944.1 |
| Crude Protein, g/kg DM | 91.5 | 565.7 | 55.5 |
| Neutral Detergent Fiber, g/kg DM | 707.5 | 602.0 | 170.6 |
| Acid Detergent Fiber, g/kg DM | 566.0 | 84.4 | 127.0 |
| Acid Detergent Lignin, g/kg DM | 64.4 | 10.3 | 29.1 |

Table 2. Post-infection mean intake (DM, OM, CP, NDF, ADF), digestibility (OM, CP), digestible matter intake (OM, CP) of kids fed hay (HAY), hay and banana (HB), hay and soya meal (HS), hay with banana and soya (HSB)

| | Diet | | | | | P-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Items | HB | HAY | HS | HSB | SEM | D | I | T | D*I | T*D | T*I |
| DM intake/g/LW 0.75 | 64.10b | 56.29c | 70.87a | 74.23a | 1.61 | 0.0001 | 0.359 | 0.0001 | 0.177 | 0.7057 | 0.876 |
| OM intake/g/LW 0.75 | 58.76b | 50.78c | 64.06a | 67.85a | 1.47 | 0.0001 | 0.28 | 0.0001 | 0.18 | 0.6232 | 0.8398 |
| CP intake/g/LW 0.75 | 5.80c | 6.08c | 19.46a | 17.15b | 0.24 | 0.0001 | 0.755 | 0.0001 | 0.146 | 0.0009 | 0.904 |
| DMO | 0.73a | 0.64c | 0.74ab | 0.78a | 0.01 | 0.0001 | 0.111 | 0.0001 | 0.704 | 0.53 | 0.7989 |
| DCP | 0.47d | 0.53c | 0.83a | 0.78b | 0.01 | 0.0001 | 0.302 | 0.0001 | 0.66 | 0.0001 | 0.9603 |
| Digestible OM intake/g/LW 0.75 | 43.24b | 32.47c | 47.89a | 51.99a | 1.4 | 0.0001 | 0.152 | 0.0001 | 0.413 | 0.5954 | 0.9232 |
| Digestible CP intake/g/LW 0.75 | 2.76d | 3.27c | 16.17a | 13.13b | 0.24 | 0.0001 | 0.31 | 0.0001 | 0.63 | 0.0001 | 0.985 |
| NDF intake/g/LW 0.75 | 27.03c | 43.71b | 51.37a | 39.08b | 1.77 | 0.0001 | 0.857 | 0.0001 | 0.407 | 0.433 | 0.9934 |
| ADF intake/g/LW 0.75 | 12.94c | 21.07a | 19.31a | 15.67b | 0.92 | 0.0001 | 0.872 | 0.0001 | 0.225 | 0.025 | 0.9754 |
| ADG, g/d | 25.18b | 26.58b | 91.19a | 118.9a | 11.06 | 0.0001 | 0.2239 | NE | 0.9824 | NE | NE |

HAY: hay distributed ad libitum

HB: hay distributed ad libitum + 1250g green banana

HS: hay distributed ad libitum + 250g soybean meal

HSB: hay distributed ad libitum + 250g soybean meal + 1250g green banana

DMO: digestibility organic matter; DCP: digestibility crude protein

D: P-value of diet

I: P-value of infection status (infected vs non infected)

T: P-value of the weeks post-infection

Table 3. Number and prolificacy of worms in abomasum of kids infected with *Haemonchus contortus* according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| | HAY | HB | HS | HSB | SEM | *P* value |
| Male[1] | 348 | 651 | 210 | 246 | 127 | 0.07 |
| Female[2] | 459[a] | 627[a] | 202[b] | 230[b] | 114 | 0.04 |
| Nematode burden[3] | 807 | 1278 | 414 | 478 | 239 | 0.06 |
| Prolificacy[4] | 6899[a,b] | 4679[a] | 14192[b,c] | 15127[c] | 2835 | 0.04 |

[1]Male, total adult male nematodes

[2]Female, total adult female nematodes

[3]Nematode burden, total adult nematodes

[4]Prolificacy, mean number of eggs produced per female adult nematode per day

Fig 1a. Post-infection OM intake of kids non-infected (NI) or infected (I) with Haemonchus contortus according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

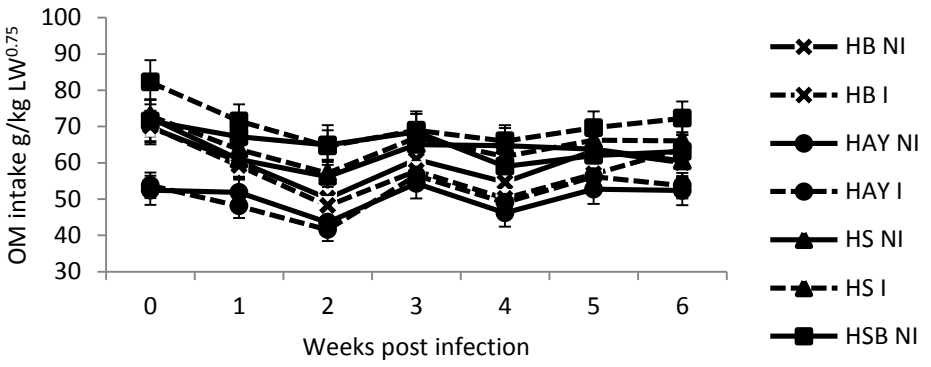



Fig 1b. Post-infection CP intake of kids non-infected (NI) or infected (I) with Haemonchus contortus according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

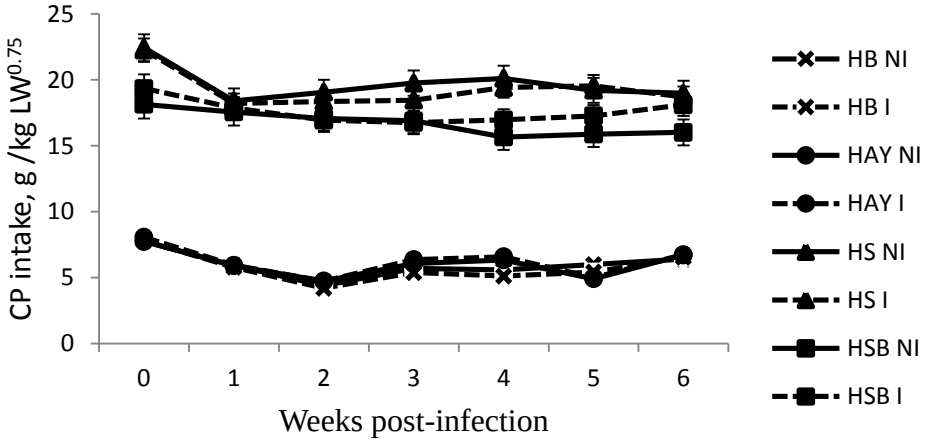


Fig 2a. Post-infection of OM digestibility of kids non-infected (NI) or infected (I) with Haemonchus contortus according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

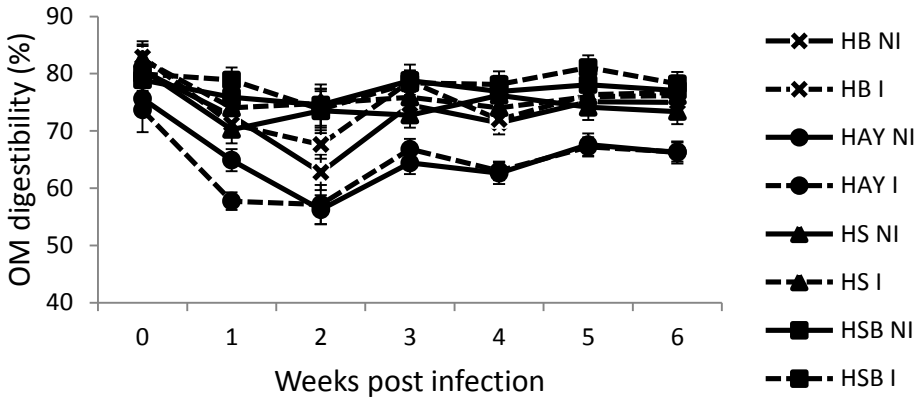



Fig 2b. Post infection CP digestibility of kids non-infected (IN) or infected (I) with Haemonchus contortus according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

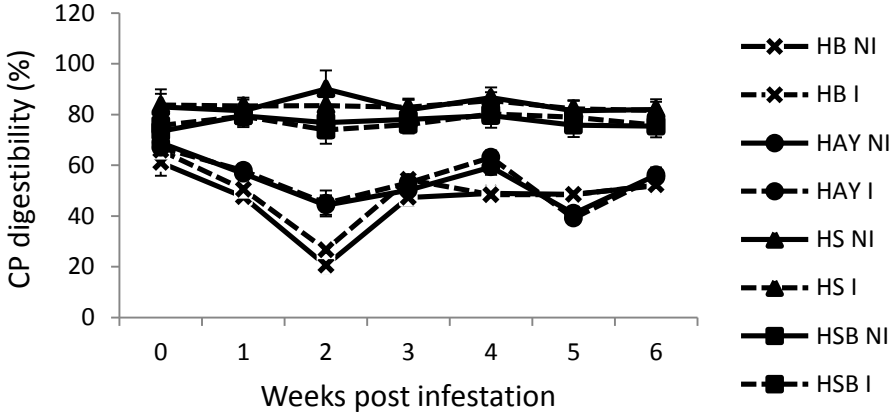


Fig 3. Post-infection fecal Eggs Counts (FEC) of kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

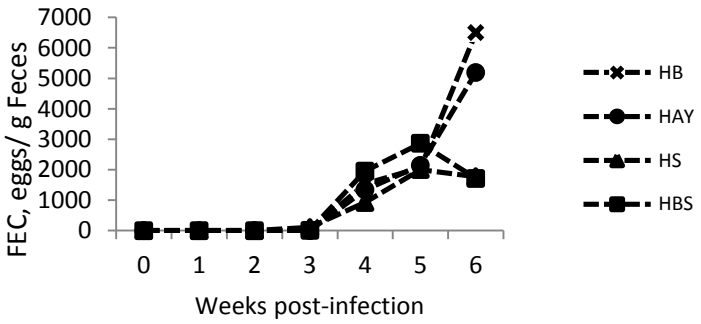


Fig 4. Average Daily Gain (ADG) of infected and non-infected kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

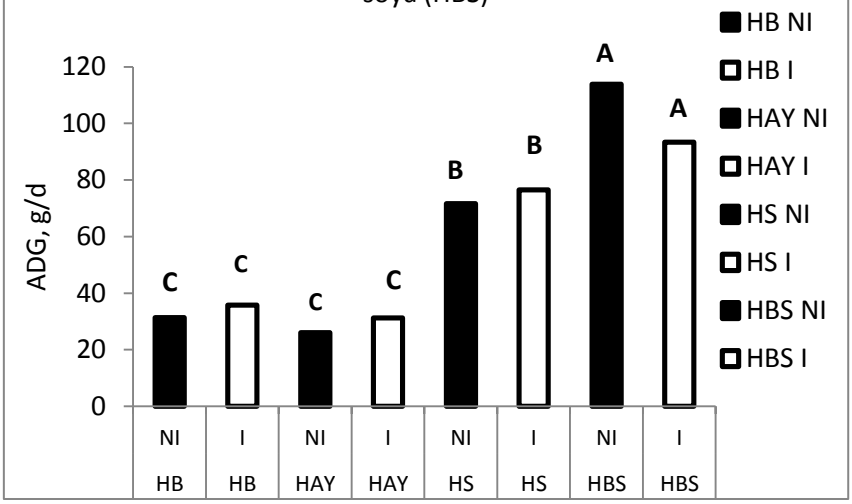


Fig 5. Packed Cell Volume (PCV, %) of non-infected and infected kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

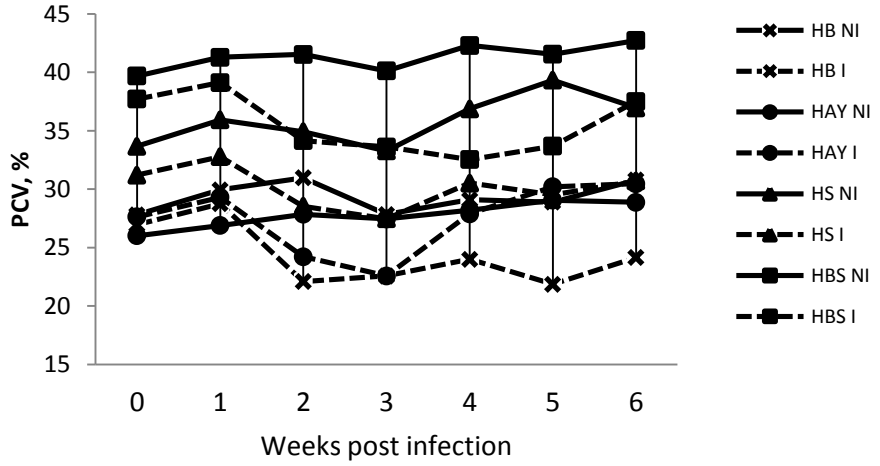



Fig 6. Red Blood Cell (RBC) of non-infected (NI) and infested (I) kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

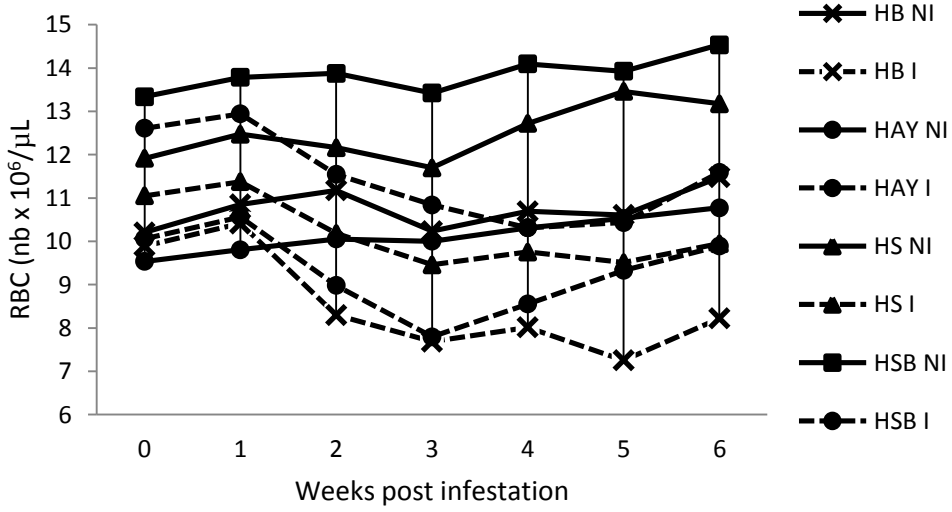


Fig 7. Blood mean corpuscular volume (MCV) of non-infected (NI) and infected (I) kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

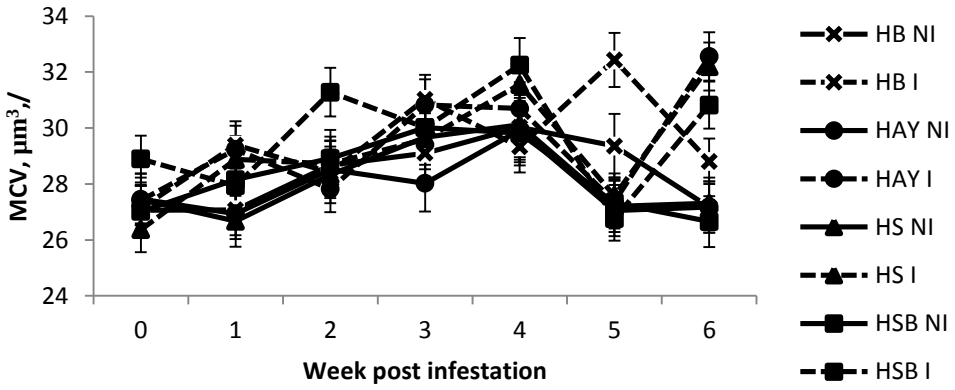



Fig 8. Blood Platelet concentration of non-infected (NI) and infested kids fed with hay (HAY), hay with banana (HB), soya meal (HS), banana and soya

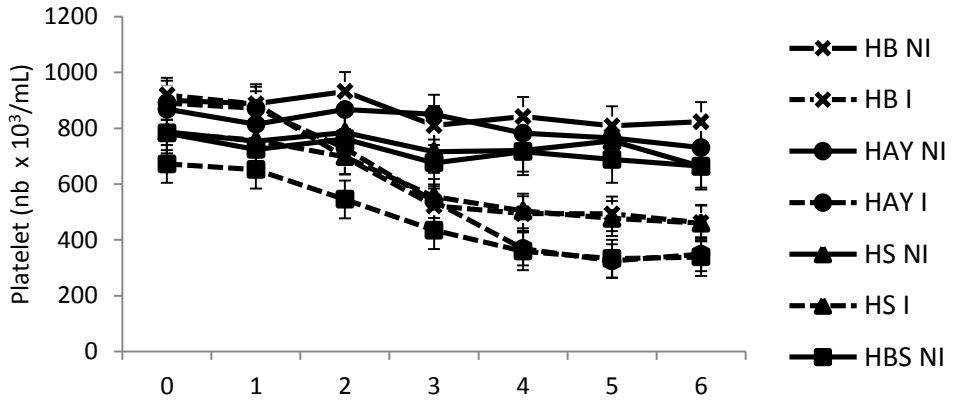


Fig 9. Blood eosinophile of non-infected (NI) and infected (I) kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

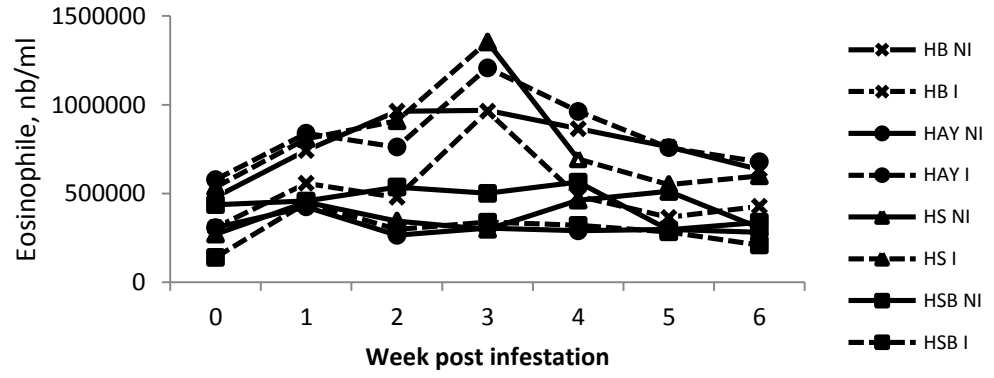



Fig 10. Blood lymphocyte concentration of non-infected (NI) and infested (I) kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

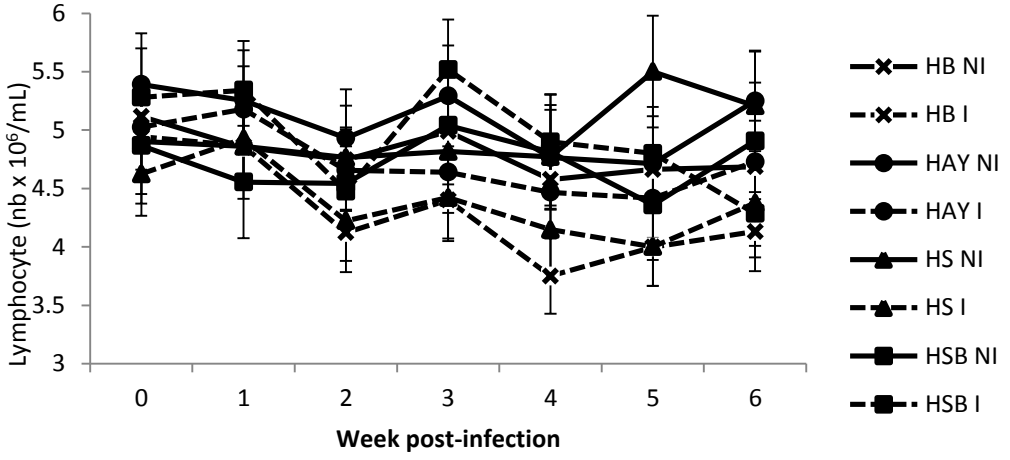


Fig 11. Blood monocyte concentration of non-infected (NI) and infested (I) kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

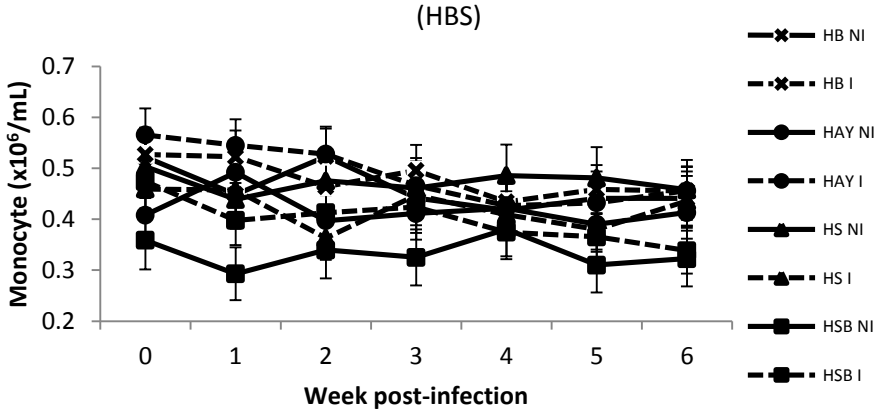



Fig 12. Blood neutrophil in non-infected and infected kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)

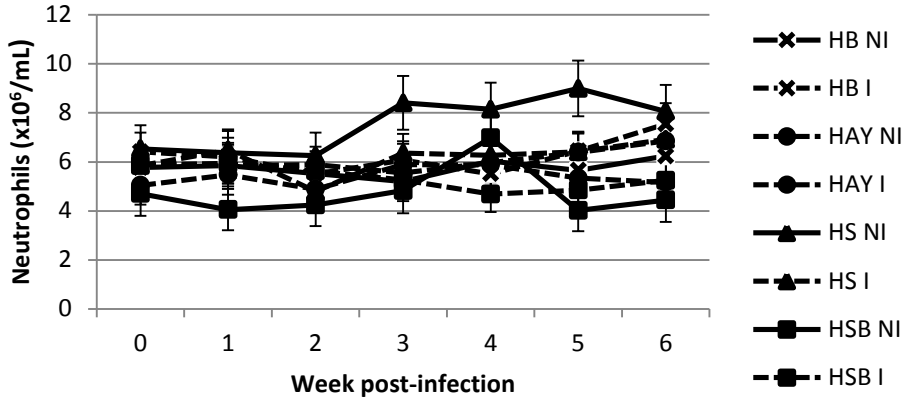
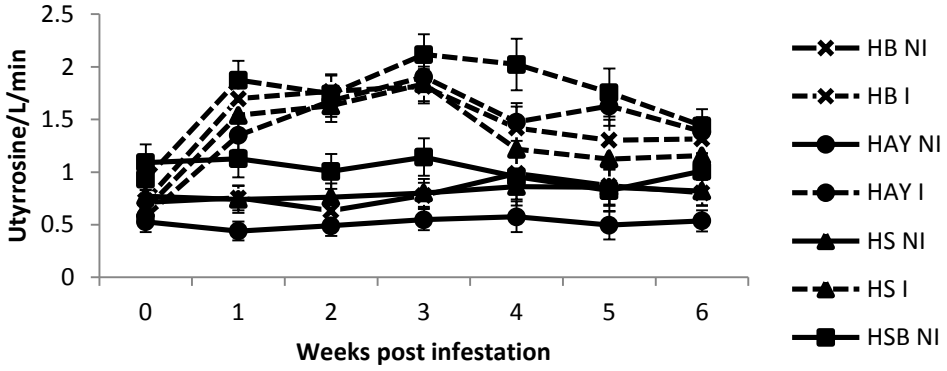


Fig 13. Blood pepsinogen in non-infected and infected kids according to the diet: hay (HAY), hay with banana (HB), soya meal (HS) and banana and soya (HBS)



## **5.3.** ARTICLE 3 : SUPPLEMENTATION WITH RUMEN-PROTECTED PROTEINS INDUCES RESISTANCE TO *HAEMONCHUS CONTORTUS* IN GOATS

**Supplementation with rumen-protected proteins induces resistance to *Haemonchus contortus* in goats**

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**Abstract**

Resistance to gastro-intestinal nematode (GIN) in small ruminant is expected to arise from protein-rich rather than from energy-rich feeds. The objective of this study was to investigate the effect of the quality of the dietary proteins on the response of Creole kid goats to *Haemonchus contortus*. Three diets were compared: no supplementation (Hay: hay *ad libitum*), Control Supplement (CS: hay *ad libitum* + 2% BW of CS at 70g of by-pass proteins/kg) and supplement enriched in Rumen Protected Protein (RPP: hay *ad libitum* + 2% BW of RPP at 139g of by-pass proteins/kg). The Faecal Eggs Counts (FEC) and the Total Faecal Eggs excreted/day (TFEC) were significantly lower in the RPP diet. No difference was found between the supplemented diets for the total number of nematodes, but the RPP diet reduced nematode prolificacy. The highest IgA responses were observed in animals with the highest nematode burden (i.e. the Hay diet compared with the CS diet). However, while the FEC and the TFEC were lower in animals feed with the RPP diet, the IgA response were

similar to those of the Hay diet. The IgA response that control GIN egg production in sheep could be one mediator of the resistance to *H. contortus* induced by by-pass proteins in goats.

**Introduction**

Small ruminant breeding, like other livestock production systems, now faces the major challenge of increasing its output with fewer resources through environmentally-friendly practices. In addition to these constraints, internal parasites among which gastrointestinal nematodes (GIN) threaten small ruminant husbandry throughout the world due to the evolution of anthelmintics resistance, the mainstay of current treatments[1,2]. Furthermore, the recent knowledge about the environmental side-effect of anthelmintic residues and the issues of public health about chemical residues in animal products reinforce the need to develop additional control strategies for sustainable production[3,4]. Three of the most promising methods which aim at enhancing the host immune response are exploitation of genetic resistance, potential vaccination and nutritional supplementation.

In small ruminants, numerous studies have shown that the nutritional status affects significantly the host response against GIN infection[5]. Indeed, it has been suggested that an improved nutritional status would fulfil the increasing needs in proteins and calories of the immune response for the production of immune cells, mediators and the repairing of damaged tissues to face invading pathogens[6-8]. In small ruminants, numerous studies have shown that nutrient supplementation improve either resilience or resistance to GIN infections[9,10]. The respective impact of metabolizable energy or proteins on the host response to GIN has long been discussed in ruminants because metabolizable energy supplementation induces metabolizable proteins supply from the ruminal microbial synthesis. Indeed, in ruminant intestinal proteins derived from both dietary proteins escaping ruminal degradation (i.e. by-pass protein) and microbial proteins synthetized in the rumen. Nonetheless, Houdijk concluded in a literature review, that it is likely that the host response would be more sensitive

to moderate metabolizable protein scarcity than metabolizable energy[11]. The poorer compatibility in term of amino acid composition of microbial proteins to the needs of the immune response compared with by-pass protein would explain this difference. Since high level of protein supplementation is not an option when production efficiency is an objective, manipulation of dietary protein that affects the quality of intestinal proteins is a key step for fine-tuning of nutritional strategies for a better control of GIN.

## Materials and Methods

All animal care, handling techniques, procedures as well as license for experimental infection and blood sampling were approved by the current law on animal experimentation and ethics (HC-69-2014-1 from the Animal Care and Use Committee of French West Indies and Guyana), 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.

### Animals and experimental design

The experiment was conducted at the experimental unit of the INRA Antilles-Guyane research center (PTEA, Tropical Platform of the Experiment on the animal, 16°12 ‘ 06.6”N 61°39 ‘ 51.6” W). The Creole goats kids (n=48, 13.34 ±2.4 kg body weight (BW); 4 months old) had experienced GIN infection at pasture before being randomly placed indoors in the individual pens corresponding to their experimental groups, 4 weeks before the experimental infection. The animals were drenched with levamisole (Polystrongle®, Coophavet, Ancenis, France, 8 mg/kg BW), toltrazuril (Baycox ovis®, Bayer healthcare, Lille, France, 20mg/kg BW) and albendazole (Valbazen® 1.9 %, Zoetis, Paris, France, 7.5mg/kg BW) and then were housed under worm-free conditions. During this period, nematode faecal egg counts (FEC) remained

at zero. Each animal was placed under one of 3 distinct diets (n=16 animals/diet): Hay (Hay ad libitum non supplemented), CS (Hay ad libitum + 2% of the BW of Control Supplement/kids), RPP (Hay ad libitum + 2% of the BW of Supplement enriched in Rumen Protected Proteins/kids) and had free access to fresh water. The composition and nutritional values of the diets is shown in Table 1. After this 4 weeks period of adaptation to the diets and to the individuals pens, a total of 10 animals/diet were experimentally infected with a single oral dose of 10,000 *H. contortus* third-stage infective larvae (L3) and 6 animals/diet remain non-infected: infected (I) and non-infected (NI) groups. The L3 were obtained 48 days before challenge from coproculture of monospecifically infected donor Creole goats with isolates previously obtained from Creole goats reared on pasture in different farms in Guadeloupe[12].

**Animal samples and measurements**

From the day of infection until the end of the experiment each animal was weighed weekly to adjust individually the offered quantities at 120% of the maximum intake capacity, according to BW changes and to measured individual growth rates. Blood samples were collected weekly by jugular venipuncture on each animal by using disposable syringes and 20-Ga needles in tubes containing an anticoagulant for complete blood counts (BD Vacutainer® spray-coated K3EDTA, Becton, Dickinson and Company, New Jersey, USA) and in dry tubes for serum analysis (BD Vacutainer®, Becton, Dickinson and Company, New Jersey, USA). Blood samples were analysed by an automaton (Melet Schloesing, MS9-5s, Osny, France). The number of circulating eosinophils was determined with Malassez cell counter[13]. Blood samples from each animal were centrifuged for 5 min at 5000 rpm. Serum samples were then frozen at -20°C until analysis. Serum pepsinogen levels were determined according to the method of Dorny and Vercruysse[14]. The biochemical parameters of the blood were determined by serum analysis (Melet Schloesing, Mscan2, Osny, France). For FEC measurements during the experimental infection, approximately 10 g of faeces were collected in plastic tubes

directly from the rectum of each animal, and transported from the experimental facility to the laboratory in refrigerated vials. The samples were individually analysed using a modified McMaster method for rapid determination and FEC was expressed as the number of eggs/g faeces[15]. The total faecal eggs excreted per day (TFEC) were calculated as follow: TFEC = FEC (eggs per gram of faeces) × weight of total faeces excreted per animal per day (g). At slaughter, the contents of the abomasum of infected animals were collected individually. The abomasum was isolated with contents to determine the worm burden. The parasites were collected, counted and sorted according to the method of Bambou et al.[16]. We collected and lyophilized the duodenum contents in order to perform aminograms. Dry matter was determined for each sample and amino acids were assayed by HPLC after hydrolysis with 6N hydrochloric acid at 110° C for 23 h. Methionine and cystine underwent performic oxidation before hydrolysis. Tryptophan was hydrolysed with barite at 110°C for 16 h. In general, the methods used for amino acids analyses were comparable with those described by Lahaye et al.[17] or Cozannet et al.[18] where more details are given. The levels of IgA anti-L3 and IgA anti-ESP was measured by indirect ELISA according to Bambou et al (2008). Crude extracts of *H. contorcus* L3 and excretory/secretory products of adults (ESP) were prepared according to Bambou, et al.[12]. In order to compare results between assays, a positive control consisting of a pool of sera containing IgA antibody was included on each plate, and OD450 of unknown samples were altered in proportion with changes of this standard. To measure the animal ingestion and digestibility, all feed offered and refused were individually weighed and sampled. During these periods, the whole of faeces excretions were individually measured and sampled. Daily, samples were proportionally pooled and preserved. The measures were realized during periods of 5 consecutive days. These measures were repeated during 8 measurements periods.

## Statistical Analysis

The parameters measured were analysed by a linear mixed model using the Proc Mixed of the software SAS (version 9.4 TS Level 1M3). Because of the skewed distributions, FEC, TFEC and eosinophils variables were logarithm transformed in Log (FEC+15), Log (TFEC+15) and log (eosinophils +1) respectively to normalized the data. The other haematological and nutritional data were square-root-transformed to normalize residual variances. The model included fixed effects of time post-infection, diets, infection status, and the significant interaction. The comparisons between means were conducted by the least squares means procedure. The significance was fixed at $P \leq 0.05$ of probability.

## Results

### Zootechnical, nutritional and parasitological parameters

The composition and nutritional values of the diets are shown in Table 1. In the non-infected groups, the highest average daily gain (ADG) was observed for supplemented animals (CS and RPP) (Fig. 1). Average Daily Gain was higher for the animals fed with the CS diet compared with the RPP ($P < 0.001$). A significant weight loss was observed during the experimental infection for animals fed with Hay (-2.85 g/day, $P < 0.001$). No difference was observed between the infected animals in the CS and the RPP groups. However, the reduction of ADG between infected and non-infected animals was higher in the CS group compared with the RPP group (-42.4% *vs.* -27.3%, $P < 0.05$).

A significant effect of the supplementation was observed for 6 out of the 8 blood metabolites measured ($P < 0.005$, Table 2). Blood Alkaline phosphatase (ALP), glucose and urea were significantly higher in the CS and the RPP groups ($P < 0.0001$). In contrast,

Aspartate amino-transferase (AST), Alanine amino-transferase (ALT) and Creatine kinase (CK) were significantly lower in the CS and the RPP groups ($P$ <0.05).

The amino acids composition of the duodenum-ileum contents of the animals was determined at 49 days post-infection (d.p.i.) after slaughtering (Table 3). No significant effect of the infection was observed (data not shown). The amounts (g/100g DM) of the different amino acids were not different between the diets except for Ala and Pro which were higher in the supplemented groups ($P$ < 0.05). The amounts of the total amino acids contents were also higher in the supplemented groups ($P$ < 0.05). The composition (%) of the total amino acids were not different between the diets except for Asp,Thr, Glu, Ala, Tyr, Trp. Compared with RPP, the percentage of Thr and Tyr were higher in Hay and CS ($P$ < 0.05). For Glu, the percentage was higher in the RPP group ($P$ < 0.05). The percentages of Asp were significantly lower in the CS group compared with the Hay and RPP groups ($P$ < 0.05). The reverse was observed for Trp, with higher percentages in the Hay and RPP groups compared with the CS groups ($P$ < 0.05).

The FEC, TFE and the number of adult male nematodes were significantly higher in the Hay and the CS groups (Table 4, $P$ < 0.05). The total number of adult nematodes (nematode burden) was significantly lower in the supplemented groups (CS and RPP, $P$ < 0.05). No difference was observed between groups for the number of adult female nematodes ($P$ > 0.05).

**Haematological and serological parameters**

The levels of serum pepsinogene increased significantly in all infected groups at 7 d.p.i. to reach a plateau until 28 d.p.i. ($P$ < 0.05, Fig. 2). Thereafter, the levels of serum pepsinogene decreased in all groups but remained significantly higher than in the non-infected groups ($P$ < 0.05). No effect of the diet was observed in the infected and the non-infected groups ($P$ > 0.05). In contrast, for PCV, hemoglobin and mean corpuscular volume (MCV), significant

interaction was observed between the diet and the infection status (infected vs. Non-infected, $P < 0.01$, Fig. 3). A transitory anemia observed only in the Hay groups from 14 until 28 d.p.i. (significant decrease of PCV and hemoglobin, $P < 0.05$) was correlated with an increase of MCV (Fig. 3). In contrast, a thrombocytopenia was observed in all the infected animals but was significantly more important in the Hay group ($P < 0.05$).

The percentage of circulating lymphocytes decreased slightly but significantly in infected animals ($P < 0.01$, Fig. 4). At 35 and 49 d.p.i. the percentage of circulating lymphocytes was significantly lower in the infected animals of the Hay group compared with the other groups infected and non-infected. A significant effect of the d.p.i. was observed for the percentage of neutrophils but no effect of the diet, the infection or their interaction was observed. The percentage of circulating eosinophils was higher in the CS group whatever the infection status ($P < 0.05$). No effect of the d.p.i., the infection status or their interaction was observed ($P > 0.05$). No significant effect was observed for the percentage of circulating basophile and monocytes ($P > 0.05$).

Following the experimental infection with *H. contortus* the levels of IgA anti-L3 and anti-ESP responses increased in all the infected animals from 7 d.p.i. to peak between 21 and 28 d.p.i., and then decreased rapidly to reach a baseline at 42 d.p.i. to the end of the infection ($P < 0.05$, Fig. 5). Infected animals in the Hay and the RPP groups had IgA responses more pronounced than those of the CS group ($P < 0.05$). The level of IgA anti-PES was higher in animals of the Hay group at 28 and 35 d.p.i. ($P < 0.05$).

**Discussion**

The positive effect of nutrient supplementation on the reduction of morbidity and mortality due to GIN infection in small ruminant has been described for a long time[19,20]. It is now hypothesized that host resistance to GIN is expected to arise from protein-rich rather than

from energy-rich feeds[11]. However, since most of the studies used over-feeding of proteins to show host resistance to GIN, the adoption of this strategy as a non-chemical control method is not yet feasible from both an economic and an environmental point of view. A fine-tuning of protein supplementation would be on the quality in term of amino acids contents. Indeed, it has been suggested that in case of metabolizable protein scarcity the effect of the quality of the feed on the resistance to GIN was significant[11]. Unfortunately, due to the ruminal activity, the manipulation of the amino acids profiles absorbed by the intestine is not an easy task. Indeed, in ruminants, the proteins arriving in the intestine are a mix of dietary proteins that escaped from the microbial degradation in the rumen (i.e. by-pass proteins), microbial proteins synthetized from dietary amino acids and endogenous intestinal proteins[21]. The profile of the microbial proteins is stable, but that of by-pass proteins and the proportion of both depends on the composition of the feed[22,23]. Thus, the objective of this study was to investigate the effect of a supplement enriched in rumen-protected proteins on the resistance of Creole kid goats to *H. contortus*. Except for Glu and Tyr, the profile of intestinal amino acids of animals fed with the RPP diet was not different from the CS and/or the Hay diet. Since the flow of amino acids arising from the abomasum is not homogenous over time, it is tempting to hypothesize that the delay between the slaughtering and the last supplement intake which was 24h in this study, was probably too important to measure the impact of the diet on the amino acids profiles in the duodenum-ileum. In the same manner, supplementation has a significant effect on blood metabolites, with no specific effect for the RPP diet. In accordance with previous studies in ruminants, urea and glucose were higher in supplemented animals[24,25]. Alkaline phosphatase levels, which is an index of skeletal and antler growth in artiodactyls and associated with osteoblastic activity[26,27], were also higher in supplemented animals which showed the higher growth rate. Aspartate Amino-Transferase, Alanine Amino-Transferase and Creatine Kinase levels were higher in the Hay groups showing the lower

growth rate for the non-infected and weight loss for the infected ones. Such results would be markers of intense liver function to meet the energy and protein requirements for maintenance and production. These enzymes have been reported to be responsible for the protein balance during the lactation peak in dairy cattle and sheep[28-30]. No significant difference was observed between the Hay and the CS diets on the FEC and the TFEC. In accordance with a previous study in this biological model, the CS diet induced resilience (i.e. same level of parasitism with maintenance of production and physiological parameters) rather than resistance (i.e. decreased level of parasitism together with maintenance of production and physiological parameters) to *H. contortus* infection[31]. In the same manner, we showed that the CS diet reduced the severity and the lengthening of the regenerative anaemia and the thrombocytopenia induced by *H. contortus*.

The study showing that depletion of CD4+ T lymphocytes significantly increased the parasitic load in a resistant sheep breed was the first to demonstrate a close association between the resistance to GIN infection and the host immune response[32]. Thereafter, significant negative phenotypic correlation has been found between blood lymphocyte counts and *H. contortus* fecundity[33]. Our results are in accordance with a previous study in lambs showing that *H. contortus* induced lymphopenia[34]. In contrast with a previous study in Creole goat[35], blood eosinophil counts were not statistically affected by the nutritional status. The high mean blood eosinophil counts in non-infected animals suggested that the one month parasite-free period was probably not enough to allow a significant decrease of the circulating eosinophils. In contrast with sheep, in goat the peripheral blood eosinophils would not play a key role in the protective response to GIN[32,36-40].

Interestingly, the FEC and the TFEC were significantly lower in the RPP diet suggesting that this diet induced resistance to the experimental *H. contortus* infection. No difference was found between the two supplemented diets (CS *vs.* RPP) for the total number of nematodes,

but the RPP diet reduced the prolificacy of the adult female nematodes. Here, the underlying mechanisms of resistance would not control the worm population establishment but rather the prolificacy of the adult female nematodes. The IgA response has been described as the major effector mechanism that control nematode egg production in sheep[41,42]. The strong association with GIN resistance and the favourable effects on growth have suggested that parasite-specific IgA might serve as a useful marker of resistance to infection[43]. In goats, the IgA response was positively correlated to FEC, suggesting that this response would be a marker of the level of parasitism[40,44]. In this study, the highest IgA responses were observed in animals with the highest nematode burden (i.e. the Hay compared with the CS diets). However, while the FEC and the TFEC were lower in animals fed with the RPP diet, the IgA response were similar to those of the Hay diet. Therefore, the RPP diet would improve the goat IgA response to control nematode egg production as in sheep.

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**Table 1**: Nutritional values of the diets

| Chemical composition | Diets | | |
|---|---|---|---|
| | Hay | CS | RPP |
| CP[1] (%) | 8.1 | 23.3 | 23.3 |
| DCP[2] (%) | 5.0 | 18.9 | 19.3 |
| PDIA/kg[3] | 24 | 70 | 139.3 |
| PDIN/kg[4] | 66 | 160.9 | 182.8 |
| PDIE/kg[5] | 51 | 126.5 | 174.5 |
| FU/kg[6] | 0.6 | 0.98 | 0.99 |
| RDS[7] (%) | - | 61.1 | 37.8 |
| RDN[8] (%) | 49 | 68.3 | 33.5 |

[1]CP : Crude Protein

[2]CPD : Digestible Crude Protein

[3]PDIA: Dietary protein undegraded in the rumen, but truly digestible in small intestine

[4]PDIN: Amount of microbial protein that could be synthetized in the rumen from the dietary nitrogen when energy and other nutrient are not limiting factors

[5]PDIE: Amount of microbial protein that could be synthetized in the rumen from Energy available in the rumen when nitrogen and other nutrient are not limiting factors

[6]FU: Feed Unit (Net Energy)

[7]RDS starch: Rumen Degradable Starch

[8]RDN nitrogen: Rumen Degradable Nitrogen

**Table 2.** Least square means of serum metabolites of Creole kids according to the dietary groups (Hay[1], CS[2], RPP[3]) infected or non-infected with an oral single dose of 10,000 third-larvae stage (L3) of *Haemonchus contortus*: Hay[1]; CS[2]; RPP[3].

| | Diets | | | | *P*-value | | | |
|---|---|---|---|---|---|---|---|---|
| | Hay[1] | CS[2] | RPP[3] | SEM | D[4] | T[5] | I[6] | D×T×I[7] |
| Alkaline Phosphatase, U/L | 125.90[a] | 317.91[b] | 396.36[b] | 37.32 | 0.0001 | 0.97 | 0.76 | 0.93 |
| Gamma-Glutamyl-Transférase, U/L | 36.95 | 42.11 | 36.98 | 3.34 | 0.5823 | 0.88 | 0.76 | 0.92 |
| Aspartate Amino-Transferase, U/L | 103.84[a] | 76.75[b] | 73.28[b] | 5.43 | 0.0009 | 0.93 | 0.82 | 0.38 |
| Alanine Amino-Transferase, U/L | 34.11[a] | 30.00[b] | 29.36[b] | 7.35 | 0.0122 | 0.01 | 0.86 | 0.19 |
| Creatine Kinase, U/L | 294.33[a] | 168.28[b] | 180.42[b] | 23.21 | 0.0016 | 0.49 | 0.88 | 0.43 |
| Creatinine, mg/L | 9.15 | 11.67 | 9.75 | 0.99 | 0.2113 | 0.39 | 0.60 | 0.55 |
| Glucose, g/L | 0.46[a] | 0.52[b] | 0.56[b] | 0.08 | 0.0001 | 0.001 | 0.79 | 0.09 |
| Urea, g/L | 0.34[a] | 0.55[b] | 0.52[b] | 0.09 | 0.0001 | 0.009 | 0.36 | 0.58 |

[1]Hay distributed ad libitum

[2]CS, Control Supplement, Hay distributed ad libitum + CS 2% of BW

[3]RPP, Supplement enriched in Rumen Protected Proteins, Hay distributed ad libitum + RPP 2% of BW

[4]D, *P*-value of diets

[5]T, *P*-value of the days post-infection

[6]I, *P*-value of the infection status (infected vs non-infected)

[7] D×T×I, *P*-value of the interaction of the 3 fixed effects

**Table 3**. Amino acids composition of the duodenum-ileum contents of Creole kids according to the diets: Hay; CS,RPP.

| Amino Acids[1] g/100g DM (% of total AA) | Diets | | | *P values* |
|---|---|---|---|---|
| | Hay[2] | CS[3] | RPP[4] | |
| ASP | 2.22 (9.6[a]) | 2.83 (10.1[b]) | 2.70 (9.6[a]) | 0.13 (0.015) |
| THR | 1.64 (7.1[a]) | 1.92 (6.8[a]) | 1.87 (6.5[b]) | 0.49 (0.05) |
| SER | 1.10 (4.7) | 1.35 (4.8) | 1.35 (4.8) | 0.15 (0.67) |
| GLU | 3.02 (13.1[a]) | 3.76 (13.3[a]) | 3.87 (13.8[b]) | 0.19 (0.05) |
| GLY | 1.22 (5.3) | 1.44 (5.1) | 1.47 (5.1) | 0.41 (0.78) |
| ALA | 1.24[a] (5.4) | 1.54[b] (5.5) | 1.58[b] (5.7) | 0.05 (0.31) |
| VAL | 1.25 (5.4) | 1.51 (5.3) | 1.47 (5.2) | 0.38 (0.34) |
| ILEU | 1.03 (4.4) | 1.26 (4.4) | 1.24 (4.4) | 0.38 (0.88) |
| LEU | 1.85 (8.0) | 2.26 (7.9) | 2.33 (8.2) | 0.27 (0.33) |
| TYR | 1.28 (5.5[a]) | 1.50 (5.3[a]) | 1.37 (4.8[b]) | 0.48 (0.05) |
| PHE | 1.05 (4.5) | 1.32 (4.7) | 1.28 (4.6) | 0.19 (0.15) |
| HIS | 0.53 (2.3) | 0.59 (2.2) | 0.69 (2.5) | 0.17 (0.51) |
| LYS | 1.62 (7.0) | 2.14 (7.5) | 2.01 (6.9) | 0.26 (0.2) |
| ARG | 1.09 (4.7) | 1.38 (4.8) | 1.37 (4.6) | 0.41 (0.85) |
| PRO | 1.51[b] (6.6) | 1.81[b] (6.6) | 1.92[b] (7.1) | 0.05 (0.61) |
| MET | 0.28 (1.2) | 0.34 (1.2) | 0.34 (1.2) | 0.48 (0.99) |
| CYS | 0.67 (2.9) | 0.81 (2.9) | 0.84 (3.1) | 0.22 (0.68) |
| TRP | 0.51[a] (2.2[a]) | 0.36[b] (1.35[b]) | 0.48[a] (1.8[a]) | 0.01 (0.01) |
| Total | 23.1 | 28.1 | 28.2 | 0.05 |

[1]Amino acids, g/100g of Dry Matter (DM) and (% of the total amino acids measured)

[2]Hay, Hay distributed *ad libitum*

[3]CS, Control Supplement, Hay distributed ad libitum + CS 2% of BW

[4]RPP, Supplement enriched in Rumen Protected Proteins, Hay distributed ad libitum + RPP 2% of BW

**Table 4**. Least squares means of the parasitological parameters of Creole kids according to the dietary groups (Hay[1], CS[2], RPP[3]) infected with an oral single dose of 10,000 third-larvae stage (L3) of *Haemonchus contortus*.

| | Diets | | | | |
|---|---|---|---|---|---|
| | Hay[1] | CS[2] | RPP[3] | SEM | *P* value |
| FEC[4] | 9.08[a] | 8.42[a] | 7.56[b] | 1.37 | 0.018 |
| TFE/day[5] | 13.46[a] | 13.06[a] | 12.02[b] | 1.17 | 0.013 |
| Male[6] | 661[a] | 211[b] | 117[b] | 345 | 0.013 |
| Female[7] | 614 | 329 | 167 | 368 | 0.082 |
| Nematode burden[8] | 1276[a] | 543[b] | 296[b] | 703 | 0.036 |
| Prolificacy[9] | 3842[a] | 8296[b] | 5243[a] | 768 | 0.048 |

[1]Hay distributed *ad libitum* non supplemented

[2]CS, Control Supplement, Hay distributed *ad libitum* + CS 2% of BW

[3]RPP, Supplement enriched in Rumen Protected Proteins, Hay distributed *ad libitum* + RPP 2% of BW

[4]FEC, back-transformed faecal eggs counts (eggs/g of faeces)

[5]TFE/day, back-transformed total excreted faecal eggs per day

[6]Male, total adult male nematodes

[7]Female, total adult female nematodes

[8]Nematode burden, total adult nematodes

[9] Prolificacy, mean number of eggs produced per female adult nematode per day

## Figures captions

**Figure 1.** Average daily gain means (ADG) of Creole kids according to the diets (Hay[1], CS[2], RPP[3]) infected with an oral single dose of 10,000 third-larvae stage (L3) of *Haemonchus contortus* or non-infected.

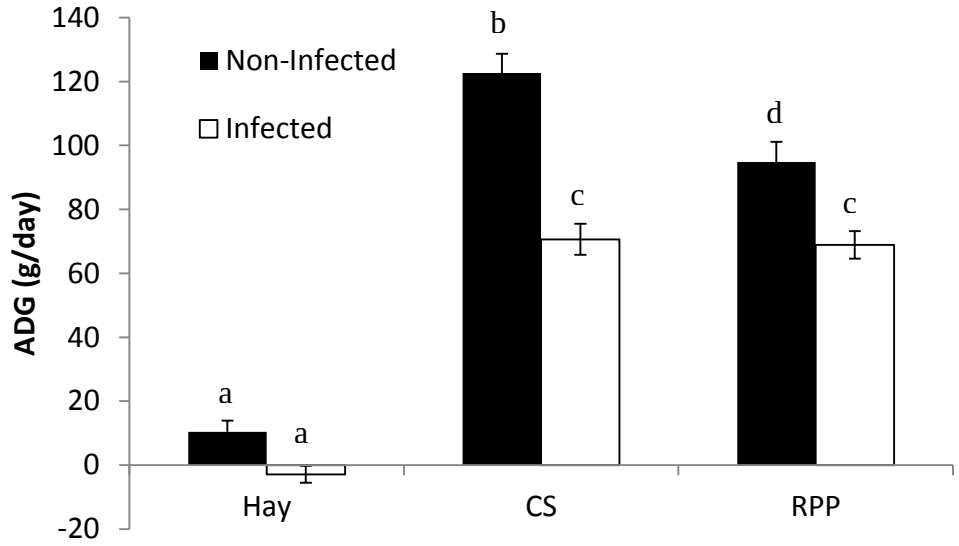
**Figure 2.** Least square means of serum pepsinogen in Creole kids according to the diets (Hay[1], CS[2], RPP[3]) infected with an oral single dose of 10,000 third-larvae stage (L3) of *Haemonchus contortus* or non-infected.

**Figure 3.** Least square means of haematological parameters in Creole kids according to the diets (Hay[1], CS[2], RPP[3]) infected with an oral single dose of 10,000 third-larvae stage (L3) of *Haemonchus contortus* or non-infected.

**Figure 4.** Least square means of blood immune cells in Creole kids according to the diets (Hay[1], CS[2], RPP[3]) infected with an oral single dose of 10,000 third-larvae stage (L3) of *Haemonchus contortus* or non-infected.

**Figure 5.** Least square means of serological IgA response against crude extract of L3 (anti-L3) antigens and against adults *Haemonchus contortus* excretion secretion products (anti-ESP) in Creole kids according to the diets (Hay[1], CS[2], RPP[3]) infected with an oral single dose of 10,000 third-larvae stage (L3) of *H. contortus* or non-infected.

**Figure 1**.

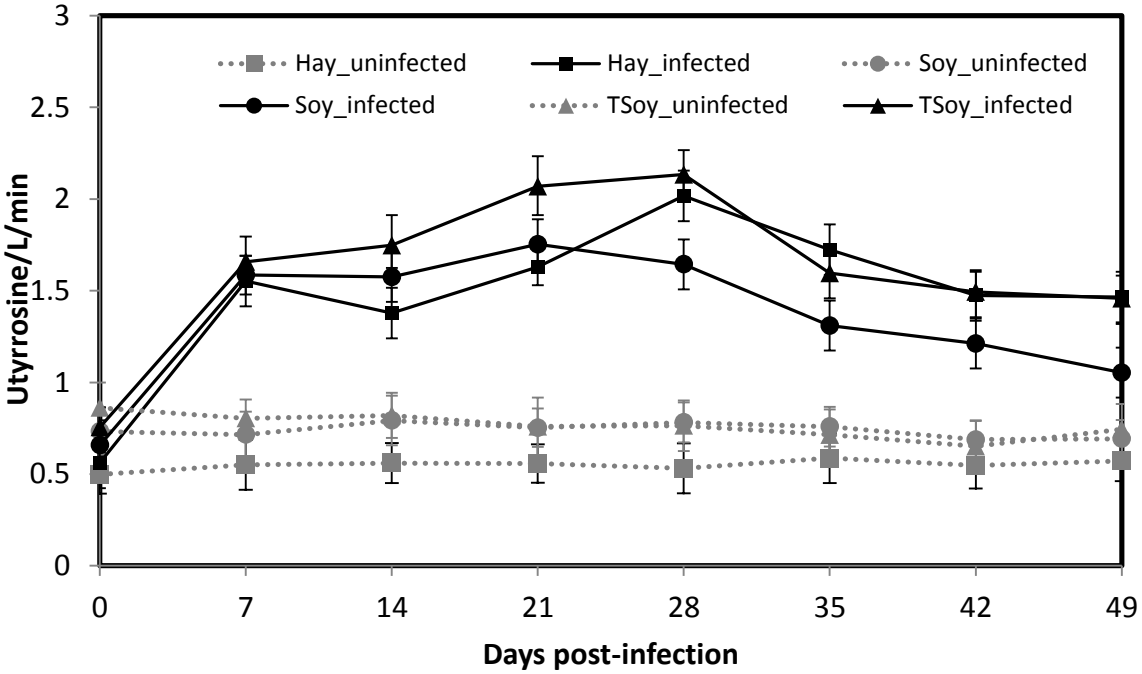



[1]Hay distributed *ad libitum* non supplemented

[2]CS, Control Supplement, Hay distributed *ad libitum* + CS 2% of BW

[3]RPP, Supplement enriched in Rumen Protected Proteins, Hay distributed *ad libitum* + RPP 2% of BW

**Figure 2.**

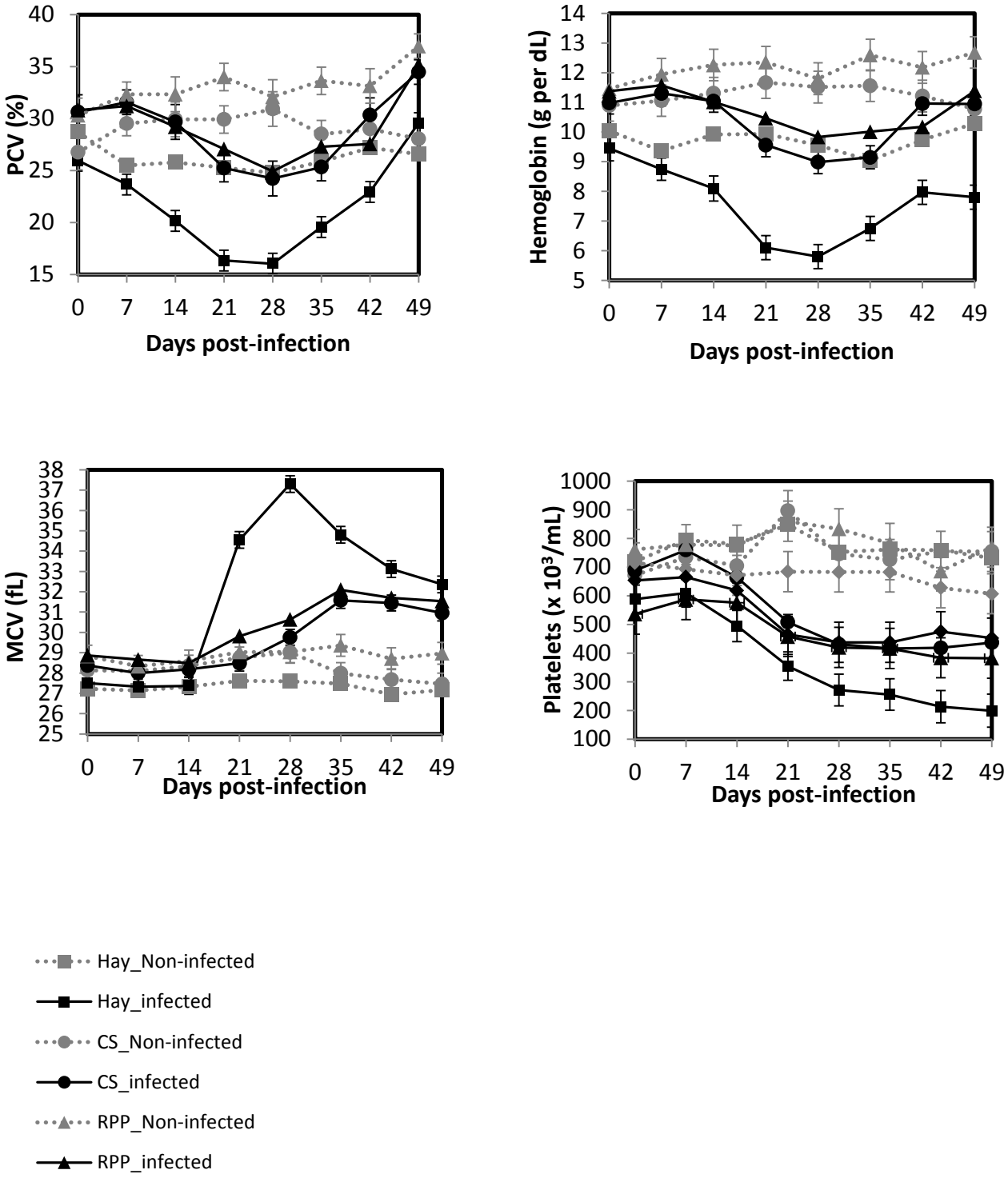



[1]Hay distributed *ad libitum* non supplemented

[2]CS, Control Supplement, Hay distributed *ad libitum* + CS 2% of BW

[3]RPP, Supplement enriched in Rumen Protected Proteins, Hay distributed *ad libitum* + RPP 2% of BW

**Figure 3.**

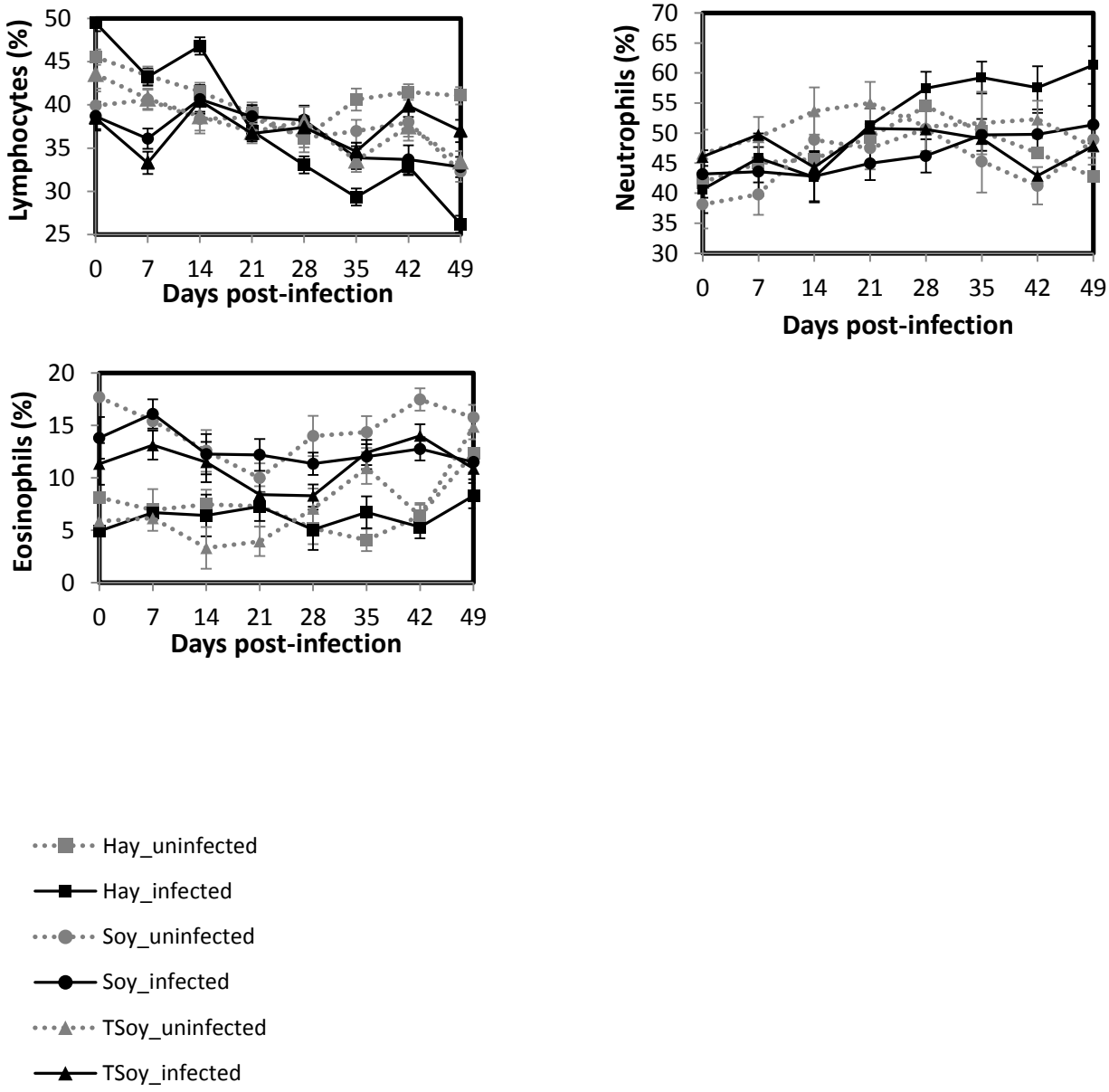



[1]Hay distributed *ad libitum* non supplemented

[2]CS, Control Supplement, Hay distributed *ad libitum* + CS 2% of BW

[3]RPP, Supplement enriched in Rumen Protected Proteins, Hay distributed *ad libitum* + RPP 2% of BW

**Figure 4.**

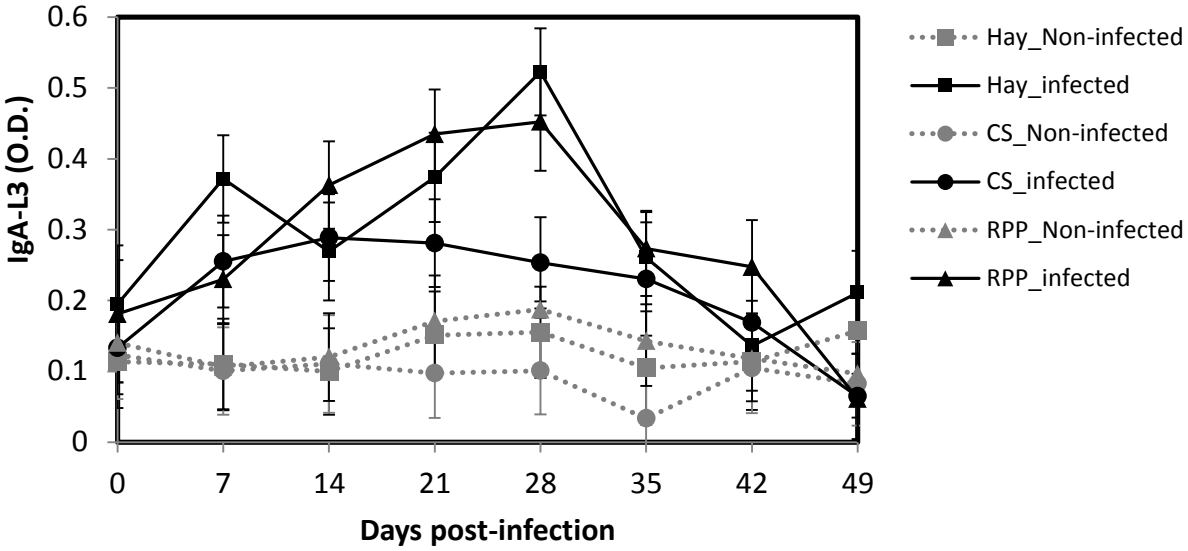


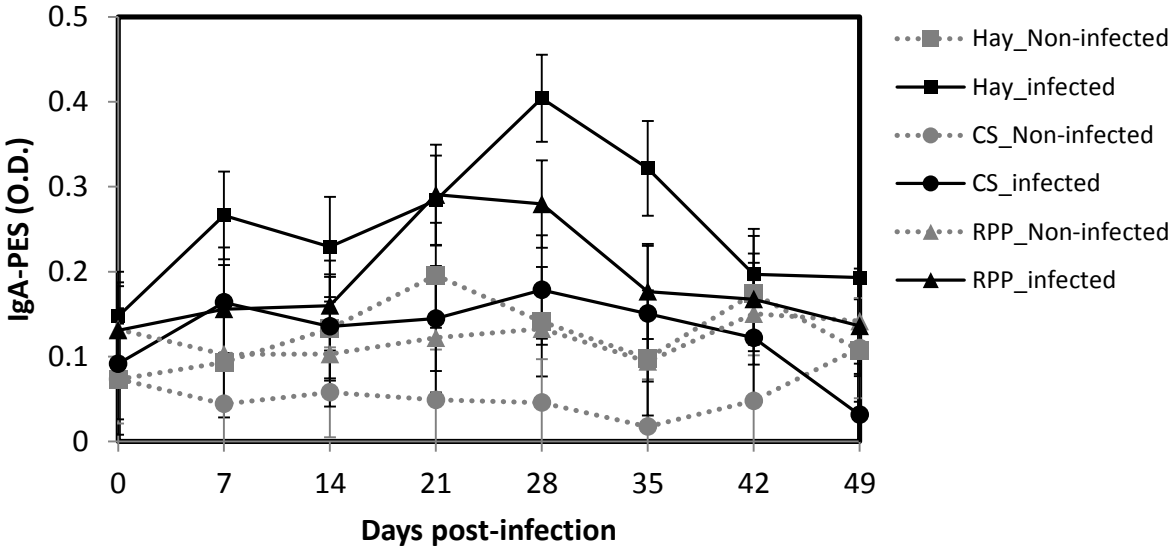

[1]Hay distributed *ad libitum* non supplemented

[2]CS, Control Supplement, Hay distributed *ad libitum* + CS 2% of BW

[3]RPP, Supplement enriched in Rumen Protected Proteins, Hay distributed *ad libitum* + RPP 2% of BW

**Figure 5.**




[1]Hay distributed *ad libitum* non supplemented

[2]CS, Control Supplement, Hay distributed *ad libitum* + CS 2% of BW

[3]RPP, Supplement enriched in Rumen Protected Proteins, Hay distributed *ad libitum* + RPP 2% of BW

## **5.4.** ARTICLE 4: ESTIMATION OF PROTEIN SYNTHESIS RATE IN DIFFERENT TISSUES OF GOATS INFECTED BY *HAEMONCHUS CONTORTUS* WITH THE DEUTERIUM OXIDE METHOD.

**Short communication**

# Estimation of protein synthesis rate in different tissues of goats infected by *Haemonchus contortus* with the deuterium oxide method.

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## Abstract

The objective of this trial was to evaluate the deuterium oxide method for the estimation of protein synthesis rate in different tissues of the biological model of goats infected with *Haemonchus contortus*. Twelve Creole bucks (7 months old; 19 kg body weight) fed with hay were used in this experiment. The daily dry matter intake, total tract digestibility and fecal egg count were measured and protein fractional synthesis rate (FSR) in abomasum, duodenum, liver and lymph nodes were estimated. A significant negative impact of infection was observed on the intakes. No effect of infestation was observed. No difference in FSR was observed between tissues.

**Keywords**: *Haemonchus contortus*, intake, total tract digestibility, fecal egg count, protein fractional synthesis rate

**Introduction**

Gastrointestinal nematode infections (GIN) can be assimilated to a nutritional disease that changes priorities in the use of rare nutrients in infected animals (Coop and Holmes, 1996). The partitioning of nutrient flows between production and immune functions in infected animals may partially explain the resistance / resilience of small ruminants to these infections. This partition could depend on the nutritional status, the genotype and the physiological stage of the animals. We can assume that resistant animals give priority to immune functions. Moreover, the classical nutrition approach arbitrarily integrates the requirement linked to resistance to environmental stresses with maintenance requirements. The partitioning of nutrients between tissues can be approached by the method of tracers including deuterium oxide ($^2H_2O$) (Gasier et al 2010). Using the deuterium, the $^2H$ rapidly equilibrates with body water and labels the cellular amino acids pools via transamination reactions. These labelled amino acids are then incorporated in proteins and tissues. The amino acid often used as a precursor of protein synthesis in the deuterium oxide method is Alanine which incorporates 4 deuterium atoms that significantly improve the accuracy of determination relative to other amino acids incorporating less deuterium atoms from $^2H_2O$. The advantages of this method are: i) the minimally invasive approach because the continuous administration of the tracer can be done orally and over long periods (especially adapted to proteins with a slow turnover rate), ii) the economic cost vs. conventional tracers, and iii) the rapid balance between the different compartments of the deuterium oxide and amino acids and therefore, most likely, a similar isotopic enrichment value between circulating amino acids and the true precursor pool (Gasier et al 2010).

The objective of this trial was to measure the effect of *Haemonchus contortus* infection on the protein synthesis in abomasum, duodenum, liver and mesenteric lymph nodes of Creole bucks with the deuterium oxide method.

## Materials and methods

### Animals, diet and experimental design

Twelve Creole bucks (7 months old; 19 kg body weight) were used in this experiment. The animals were placed in individual metabolic cage for the duration of the experiment (4 weeks) and fed with medium quality hay. At the end of the first week of adaptation to the diet, half of the animals were artificially infected orally with a dose of 10,000 *H. contortus* infective larvae. At the end of the 4 weeks of adaptation to the diet, all the animals received an intrajugular injection of $^{2}$H2O (99%) at 0.9% NaCl maintained at a temperature of 37°C prior injection. A volume of 100 mL was injected during 5-7 min to avoid vein damages. This volume was calculated taking into account an average weight of 15-20 kg per animal, a total body water volume of 60% of body weight (range between 50-80%) and an isotopic enrichment of the desired body water of about 1% (corresponding to an isotopic enrichment of free plasma alanine of about 3.7%).

### Chemical analyses and analytical procedures

Hay samples were milled through a 1mm screen (Reich hammer mill, Haan Germany) prior to analysis. Organic matter (OM) and N analyses were performed according to AOAC (1990), Methods 923.3 and 992.15, respectively, for OM and N. Crude protein (CP) was estimated as Nx6.25. Cell wall components (neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL)) in samples were determined as described by Van Soest et al. (1991) using a sequential procedure (AOAC, 2006, Methods 200.04 and 973.18,respectively, for NDF and ADF + ADL).

Plasma were thawed overnight, deproteinised (1 mL) with sulphosalicylic acid (10%, w/w) and centrifuged then at 2500 g for 10 min at 4°C. The supernatant was purified on AG50 X8

H+ resin (Bio-Rad, Hercules, CA, USA) and an N-tert-butyl-dimethyl-silyl amino acid derivative was prepared (Calder and Smith, 1988). Tissues were thawed overnight and a small amount (between 5-10 mg according to the protein content of the tissue) was hydrolyzed with HCl 6N during 24h at 110°C. After eliminating the liquid by centrifugal evaporation, the residual amino acids solid were derivated with the same product as the plasma proteins. Alaline was assayed by chromatography-mass spectrometry (GC-MS) analysis.

**Sampling, measurements and calculation**

The amounts of proposed and refused hay were weighed daily. The daily amounts of excreted feces were weighed over 5 consecutive days during the fourth week of measurement. Samples of feces were collected from the rectum at 14 and 21 days post-infection to determine Fecal Eggs Counts (FEC) and confirm the health status of the animals.

Blood samples were taken at 0, 30 min, 1h, 2h, 4h, 6h and 10 h after injection of the 100ml $^{2}H_2O$, in anticipation of the estimate of the Fractional synthesis rate (FSR). The latter is the rate at which $^{2}H$-labeled alanine is incorporated into muscle proteins relative to the total abundance of the alanine pool per unit of time. All the animals were slaughtered 10h after the end of $^{2}H20$ injection. Tissues (20 grams) were sampled from the digestive tract and from the liver (always the same lobe and anatomical region). These samples were taken quickly (5 to 10 mm) after slaughter. Once removed, the tissues were cut into small cubes (1 cm x 1 cm), placed in a tube and frozen at -80°C.

The daily dry matter intake was measured by difference in the amounts of feed offered and refused. The digestibility of the material was estimated as follows: (ingested hay – excreted feces)/ingested hay)

The fractional synthesis rate of protein (FSR, % d) in tissues was estimate as follows:

FSR = 24 x 100 x [(atom percentage excess of labeled Ala in tissue, %) / (atom percentage excess labeled Ala in plasma, %) × Time between infusion and tissue sampling]

**Statistical analysis**

Statistical analyses were performed using the mixed procedure of SAS (SAS, 2008). Regarding the indicators of the intake, total tract digestibility and fecal egg count, the model included infection status (n= 2) as fixed effects and animal as random effect. Concerning the fractional synthesis rates, the model included infection status (n= 2), tissues (n=4) and interaction status x tissues as fixed effects (n=8) and animal as random effect.

**Results**

The chemical composition (g/kg DM) of the experimental hay grass was: 887, 79, 777, 429 and 74 g for Organic matter, Crude protein, Neutral detergent, Acid detergent and Acid detergent lignin respectively. The main results of the trial are summarized in Table 1. Marked water injections were given between 5 and 7 min and no abnormal behavior was observed in the animals. The DM intakes of infected bucks were 24% lower than those of non-infected bucks. No difference was registered for DM total tract digestibility. As expected, fecal egg count was higher in infected bucks whereas non-infected bucks were parasite-free.

The kinetic curve of labelled alanine in the blood serum shows that a plateau is reached after 30min post injection and is maintained over about 10 hours, at the time of slaughter of animals (Figure 1.). The plasma alanine free concentration varied greatly between bucks as illustrated by the high standard errors.

No significant difference was registered for alanine FSR whatever the tissue taken into account.

**Discussion**

The lower intake measured in infected bucks compared to non-infected ones is a result quite frequently observed in the literature, although it is not a systematic trend (Ceï et al, 2018). Indeed, Sykes (2010) indicates that anorexia is mainly observed with naïve young animals probably due to the activation of their immune system. This anorexia disappears in adults previously infected. Moreover, high infection level is an aggravating factor causing anorexia. Yet, in this experiment the animals were young bucks whose immune systems had already been activated by *H. contortus* at pasture. Our results therefore confirm the wide variability observed in the literature on this criterion. The absence of *H. contortus* effect on total tract digestibility is also consistent with the broad variability found in the literature, although the meta-analysis by Ceï et al (2018) indicates as a general tendency a decrease of total tract digestibility with gastrointestinal nematode infestations. This decrease is attributed to a change in the peristalsis of the digestive tract accelerating the rate of diet transit.

The FSR estimates in this trial are in agreement with values found in the literature for ruminants (Bermingham et al, 2007; Lescoat et al 1997). In a literature synthesis, Lescoat et al (1997) report FSR variation from less than 1 %/day for some muscle tissues to more than 100%/day for the gastrointestinal tract tissues. Comparisons of FSR estimates in the literature should, however, be analyzed with caution given many potential sources of variation including methodologies (choice of the precursor pool determines the range of FSR values, amino acid labeled…), physiological state of the animal, feeding intake and animal characteristics. For this reason, Lobley et al (1996) termed FSR measurements as 'semi-quantitative' providing data on the relative effects of experimental factors of variation studied in a trial. Nevertheless, contrary to our working hypotheses and the literature, this trial did not reveal significant differences in FSR between tissues. As an illustration, the rate of protein synthesis is much higher for splanchnic organs than peripheral tissues ( e.g., 22% $d^{-1}$, 50% $d^{-}$

[1], 3–4% $d^{-1}$ in liver, duodenum and muscle of sheep; Lobley et al.1994) (e.g. sheep muscle; Lobley 1993). Furthermore, *H. contortus* leads to: 1) aggression of the abomasal epithelium and consequently increased repairing mechanism; 2) an immune response which should result in stronger protein synthesis in the liver and abomasal lymph nodes. It was therefore expected that for these three tissues (abomasum, liver and lymph nodes), the FSR would be higher in infected animals compared to non-infected. Indeed, amino acid requirements of the intestinal, hepatic and immune tissues increase with infection (Sykes et al. 1988). The large individual variability observed between the bucks for FSR combined with the low number of animal per treatment could explain the absence of effect of the experimental infection on FSR. The use of alanine as labeled amino acid could also explain this result. Houdijk (2012) indicates that various immune cells prefer alanine and glutamine as energy sources rather than glucose, and that glutamine is extensively metabolized in many immune cells including thymocytes, lymphocytes, neutrophils and macrophages.

**Conclusion**

No effect of *H. contortus* infection was observed on the protein synthesis rate of the tissues analyzed in this study. This result may be explained by the high individual variability and limitations of the method used.

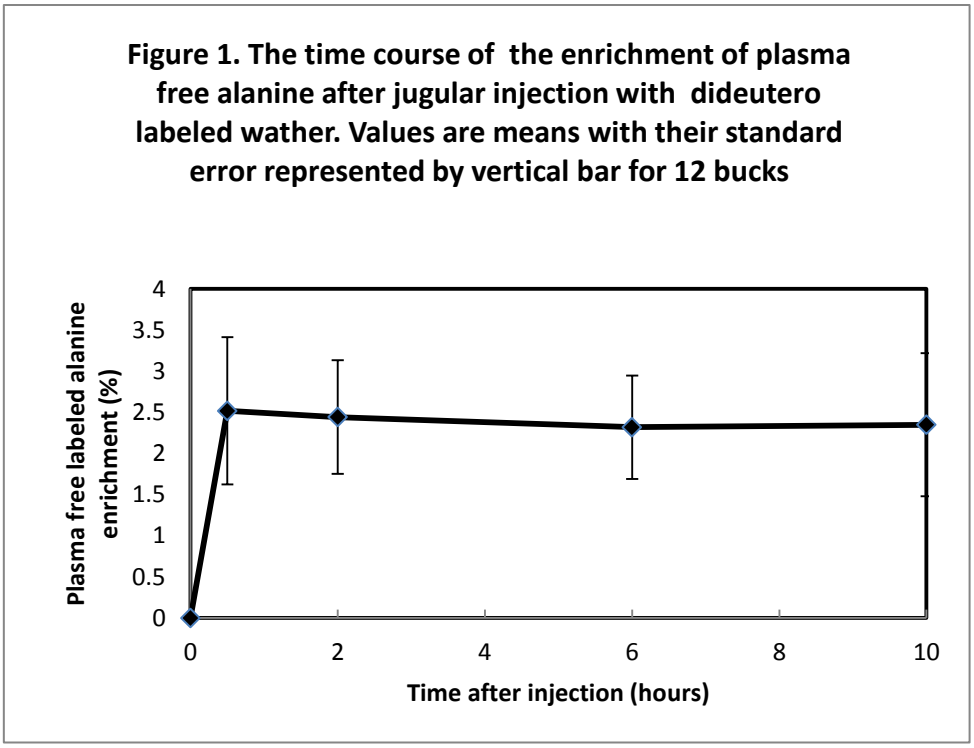


**Table 1**. Effect of *Heamonchus contortus* artificial infestation on intake, digestibility, fecal egg count, alanine fractional synthesis rate in some tissues (abomasum, duodenum, liver, mesenteric nodes) of Creole bucks fed with hay grass from a natural grassland.

| | Non-infected | Infected | SEM | *P*-values |
|---|---|---|---|---|
| Live Weight of kids | 16.9 | 16.9 | 1.04 | 0.9600 |
| Dry Matter intake, g/kg $LW^{0.75}$ | $52.5^{b}$ | $42.3^{a}$ | 2.75 | 0.0001 |
| Dry Matter digestibility (%) | 0.68 | 0.69 | 0.013 | 0.210 |
| Fecal Egg Count/ g Faeces | 0 | 2707 | 1004 | 0.0005 |
| Alanine fractional synthesis rate(%/$d^{-1}$) | | | | |
| Abomasum | 55.1 | 37.8 | 11.78 | 0.311 |
| Duodenum | 59.3 | 39.8 | 12.17 | 0.268 |
| Liver | 27.2 | 29.2 | 11.78 | 0.908 |
| Mesenteric Lymph nodes | 62.4 | 49.1 | 11.78 | 0.433 |

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# DISCUSSION ET PERSPECTIVES

### 6.1. Rôle de la nutrition sur la réponse des caprins aux NGI : Résilience ou Résistance ? Energie ou Protéine ?

Les effets bénéfiques d'une supplémentation à la fois en énergie et en protéines sur la réponse des petits ruminants aux infestations par les NGI sont connus depuis les années 1930 (Houdijk, 2012). L'hypothèse d'une amélioration des mécanismes de défense et de réparation grâce à l'apport supplémentaire de nutriments au moment où l'organisme subit les effets délétères du parasitisme fait consensus. Cependant, les rôles respectifs de l'énergie et de la protéine, sont encore discutés particulièrement chez les ruminants avec leur physiologie digestive complexe. Par ailleurs, les réponses animales varient en fonction des espèces, des races, voire des lignées. En réponse à une supplémentation, certaines études mettent en évidence de la résistance et d'autres de la résilience aux infestations à la fois chez les caprins et les ovins (Bambou et al., 2011; Garate-Gallardo et al., 2015; Knox and Steel, 1999; Liu et al., 2005; Louvandini et al., 2006; Wallace et al., 1998). Dans les deux cas, les mécanismes sous-jacents restent à caractériser.

Le parasite « modèle » de nos travaux, *H.* contortus, l'un des nématodes les plus virulents chez les petits ruminants, a été choisi à cause de sa prévalence en zone tropicale humide pouvant atteindre 100% en Guadeloupe (Aumont 1997). C'est un nématode hématophage qui se nourrit du sang de son hôte et qui par conséquent induit une anémie. L'hématocrite est l'une des mesures les plus utilisées pour évaluer le niveau d'infestation des animaux. Une méthode d'estimation de l'anémie par observation de la muqueuse oculaire, la méthode FAMACHA©, a d'ailleurs été développée pour permettre aux éleveurs d'objectiver le niveau d'infestation de leurs animaux et ainsi leur permettre de ne traiter que les animaux fortement infestés (Vatta et al., 2001). De fortes corrélations phénotypiques et génétiques entre l'hématocrite et l'OPG ont été mises en évidence par de nombreux travaux (Amarante et al., 1999; Baker et al., 2001; de la Chevrotière et al., 2012; Heckendorn et al., 2017; Rout et al., 2011). Ce paramètre sanguin est utilisé comme l'OPG pour mesurer la résistance et la résilience des animaux notamment lorsque *H. contortus* est présent. Dans l'essai 1, nous avons montré que *H. contortus* induisait une anémie régénérative et une thrombopénie chez les caprins Créole (Cériac et al., 2017). La sévérité ainsi que la durée de ces troubles physiopathologiques étaient réduites par un régime alimentaire enrichi à la fois en énergie et en protéines sans affecter la charge parasitaire mesurée via les OPG. Ces résultats suggèrent que la résilience à l'infestation *H. contortus* serait au moins en partie associée à une augmentation de la capacité de synthèse de la moelle osseuse pour compenser les pertes en

globules rouges. Par ailleurs, cette diminution du nombre de plaquettes, cellules impliquées dans le système hémostatique de l'hôte, avait peu été décrite dans la littérature (Andronicos et al., 2014). Ce résultat, appuyé d'une étude *in vitro* caractérisant une molécule exprimée par *H. contortus* capable d'inhiber l'agrégation et l'adhésion plaquettaire, suggère que ce parasite hématophage aurait développé la capacité de manipuler le système hémostatique de son hôte (Crab et al., 2002). Cependant, il a également été montré qu'une supplémentation en énergie et en protéines induisait plutôt de la résistance, c'est-à-dire une charge parasitaire et des conséquences physiopathologiques moindres (Bambou et al., 2011; Louvandini et al., 2006; Nnadi et al., 2009). Des différences entre stades physiologiques des animaux de ces différentes études pourraient expliquer ces différences. Une méta-analyse récente montre en effet qu'un meilleur statut nutritionnel à la fois en protéine et en énergie induit de la résilience à la fois chez les ovins et les caprins (Ceï et al., 2018). Néanmoins, leur contribution à la résistance n'a pas été mise en évidence.

L'objectif du deuxième essai conduit au cours de ce travail était de caractériser l'impact du niveau d'apport d'énergie et de protéines sur les réponses productives et immunitaires des chevreaux (Cériac et al. en préparation). Il n'a pas été mis en évidence d'interaction entre les régimes alimentaires et l'infestation expérimentale par *H. contortus*. Les régimes riches en protéines induisaient de la résilience vis-à-vis de l'infestation et aucun effet de la supplémentation en énergie n'a été observé. Cependant, ce résultat est à nuancer puisque la disponibilité en protéine du régime témoin (foin seul) était supérieure à celle du régime supplémenté en énergie (foin + bananes) puisque les animaux ingéraient de la banane fraiche au détriment du foin. En effet, aucune différence de vitesse de croissance n'a été observée entre ces deux lots expérimentaux. Néanmoins, de manière intéressante, nous avons montré que des OPG identiques reflétaient des charges parasitaires différentes, notamment en nématodes adultes femelles, et par conséquent des prolificités (i.e. le nombre d'œufs excrétés par femelle et par jour) différentes. Les populations de vers adultes femelles étaient inférieures et leurs prolificités supérieures chez les animaux dans les lots supplémentés en protéines. La grande variabilité des résultats pour la population totale (femelles et mâles) explique probablement qu'il n'y ait pas d'effet significatif mais uniquement une tendance ($P = 0.06$ pour 7 animaux par lot). Ces résultats suggèrent que ces régimes pourraient permettre aux animaux de limiter l'installation des nématodes mais pas leur prolificité, qui augmente quand la population diminue. Cette prolificité densité-dépendante a déjà été décrite dans la littérature pour d'autres nématodes (Paterson and Viney, 2002; Tompkins and Hudson, 1999;

Viney, 2002). Cependant, les études concernant *H. contortus* n'ont pas mis en évidence cette relation entre densité de population et prolificité (Coyne et al., 1991a; Coyne et al., 1991b). Un effet indirect de la supplémentation en protéines sur la réponse immunitaire de l'hôte pourrait néanmoins expliquer nos résultats. En effet, de nombreuses études chez les ovins montrent une corrélation négative entre la réponse IgA contre les NGI, la longueur des femelles et leur prolificité (Stear et al., 2004; Stear and Bishop, 1999; Strain and Stear, 2001). Chez les caprins, les IgA ne seraient pas les médiateurs de cette réponse (Bambou et al., 2008; Bambou et al., 2011; McBean et al., 2016).

### 6.2. Rôle de la nutrition protéique sur la réponse des caprins aux NGI : qualité ou quantité ?

La solution qui consisterait à proposer des itinéraires techniques aux éleveurs avec une supplémentation en protéines n'est satisfaisante ni du point de vue économique, ni du point de vue environnemental. Cette stratégie pourrait être affinée en proposant des périodes cibles (e.g. au sevrage, autour de la parturition) qui correspondent à des stades physiologiques plus sensibles aux effets délétères du parasitisme. Il s'agirait d'utiliser la supplémentation en préventif. Par ailleurs, il a été montré que la supplémentation pouvait également avoir des effets curatifs en réduisant significativement les effets délétères du parasitisme (Cei et al., 2017).

Une alternative consiste à affiner nos connaissances sur les effets de la supplémentation protéiques en termes de qualité pour proposer des itinéraires techniques plus efficients. Comparées aux protéines by-pass, les protéines microbiennes sont moins compatibles aux besoins de la réponse immunitaires en termes de profil d'AA (i.e. qualité des protéines) (Houdijk, 2012). La manipulation des protéines de la ration pour impacter la qualité des protéines intestinales apparait comme une piste à explorer pour un meilleur contrôle des infestations par les NGI. Ainsi, l'objectif du troisième essai était de mesurer l'effet d'une ration enrichie en protéines protégées de la dégradation ruminale (RPP pour Rumen protected protein) (Cériac et al. soumis en juillet 2018). Les vitesses de croissance étaient inférieures chez les animaux alimentés avec le régime RPP comparés au régime témoin (régime iso-azote et énergie). Nous avons montré une diminution des OPG mais pas de la charge parasitaire (i.e. nombre de parasites adultes mâles et/ou femelles) chez les animaux alimentés avec la ration RPP, suggérant l'induction d'une résistance vis-à-vis de *H. contortus* avec un effet sur la prolificité. L'un des mécanismes sous-jacents pourrait être une augmentation de la réponse en IgA contre *H. contortus*.

### 6.3. Interactions supplémentation protéique, parasitisme et répartition de flux de nutriments

La répartition des flux de nutriments entre fonctions productives (i.e. croissance, lait, fibres) et adaptatives (i.e. réponse immunitaire) pourrait, au moins en partie, expliquer les différences observées sur les réponses de résistance et de résilience des petits ruminants vis-à-vis des infestations par les NGI. Ces arbitrages physiologiques dépendent de nombreux facteurs, notamment le génotype, le stade physiologique et le statut nutritionnel. Nous avons montré qu'il serait possible de formuler des rations qui permettraient d'induire de la résistance vis-à-vis des NGI. Cependant, la diminution de la vitesse de croissance observée souligne la nécessité d'explorer finement les arbitrages de répartition des flux de nutriments pour affiner cette stratégie. L'essai 4 a été conçu dans cet objectif. Contrairement aux hypothèses de travail, nous n'avons pas mis en évidence de différences de synthèses protéiques entre les différents tissus étudiés. A notre connaissance, il n'y a pas d'études dans la littérature réalisée avec *H contortus* permettant de situer ces premiers résultats. Cette étude exploratoire ne permet pas de conclure. La forte variabilité individuelle n'a pas permis de dégager d'effets significatifs. Par ailleurs, la méthodologie de l'eau lourde utilisée pour sa simplicité a pu manquer de précision pour les mesures effectuées.

### 6.4. Perspectives

L'objectif principal de ce projet était d'analyser l'impact du statut nutritionnel (énergie et protéine) sur les réponses productives et adaptatives des chevreaux aux infestations par les SGI. Les principales conclusions sont que l'énergie tout comme les protéines impactent la résistance et la résilience aux SGI. La démonstration de l'impact de l'énergie par la voie expérimentale est compliquée du fait des interactions cellulaires entre le métabolisme énergétique et protéique. Il n'est par ailleurs pas exclu que les cellules mobilisées dans les réponses immunitaires utilisent préférentiellement des AA comme source d'énergie. Les réponses animales observées seraient fortement influencées par le niveau nutritionnel des animaux, la surnutrition pourrait « effacer » et/ou masquer l'effet de la qualité des nutriments alimentaires.

Nous avons montré : i) qu'il n'y a pas de réduction significative de l'ingestion et la digestibilité des caprins infestés par *H. contortus* confirmant la grande variabilité rapportée dans la littérature ; ii) que la surnutrition énergétique n'améliore pas la résistance des chevreaux à *H. contortus*. Ce résultat confirme la difficulté expérimentale de découpler les

effets des nutriments énergétiques et protéiques ; iii) que la surnutrition protéique renforce la résilience des caprins à *H. contortus* ; iv) que l'augmentation du ratio protéines by-pass alimentaires/protéines microbiennes accroît la résistance des chevreaux à *H. contortus* confirmant des résultats de la littérature. Des analyses en cours devraient préciser le profil d'AA concernés. L'accumulation de connaissances sur l'effet des nutriments sur la résistance aux SGI est tributaire de progrès à réaliser dans la conception des protocoles expérimentaux. Des essais sur un nombre limité d'animaux avec des mesures fines pourraient y contribuer. Les travaux analytiques sur l'effet de la qualité des protéines devraient être construits autour d'approches ciblées sur des profils AA plutôt que d'ingrédients protéiques pour lesquels la composition en AA varie fortement. L'accumulation de connaissances sur la partition de flux de nutriments entre fonction de production et réponse immunitaire permettra de mieux comprendre l'impact des stratégies nutritionnelles. Des développements méthodologiques, comme celui initié dans ce projet, avec marqueurs ouvrent des pistes prometteuses pour l'avenir.

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