Contribution of gut microbial lysine to liver and milk amino acids in lactating does

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The contribution of microbial amino acids through caecotrophy to tissue protein metabolism was investigated in lactating does. Attempts were made to vary microbial supply through a dietary antibiotic, Zn bacitracin, and to vary tissue demand through manipulation of litter size. Three groups of eight New Zealand does were fed different experimental diets from day 28 of pregnancy to day 26 of lactation. The control group received the basal diet formulated to meet requirements with grass hay, wheat, soyabean meal and barley grain. The second (no antibiotic) group and the third (bacitracin; BAC) group ingested the basal diet supplemented with ammonium sulfate (5 g/kg), initially unlabelled (day 1 to day 8) then labelled with 15N (day 9 to day 30), while the BAC diet was also supplemented throughout with antibiotic (Zn bacitracin; 100 mg/kg). From just after birth each group of does was subdivided into two groups, each of four females, with the litter size either five (LS5) or nine (LS9) pups. The 15N enrichment in liver, milk and caecal bacteria amino acids was determined by GC-combustion-isotope ratio MS. All amino acids in bacterial protein were enriched with the (15NH4)2SO4 treatment, with lysine 15N enrichment significantly greater in caecal bacteria (0·23 (SE 0·0063) atom % excess (ape)) than in liver (0·04 (SE 0·0004) ape) or milk protein (0·05 (SE 0·0018) ape), confirming the double origin (bacterial and dietary) of tissue lysine. The contribution of microbes to tissue lysine was 0·23 (SE 0·006) when milk protein was used as reference.

Caeotrophy: Rabbit does: Microbial lysine

Although the contribution of microbially derived essential amino acids to absorptive supply has been well documented in ruminants, fewer data are available for non-ruminants. In pigs, recent reports suggest that 10% of lysine requirements are derived from intestinal bacteria. In rabbits, caecotrophy is a crucial physiological process that provides a source of high-quality protein to the animal by recycling microbial biomass synthesised in the caecum. Therefore, protein nutrition in rabbits and others lagomorphs is impacted by factors that alter caecal fermentation. Quantification of the microbial contribution to amino acid supply in rabbits is difficult. However, its importance is determined normally by fitting a neck collar to prevent soft faeces ingestion, but this methodology can affect both animal behaviour and digestive physiology.

Material and methods

Protocols and animal handling through the present experiment were approved by the Comité Etico del Servicio de Biomateriales of the University of Zaragoza.

Animals

Twenty-four New Zealand White multiparous does with a mean initial body weight of 4·3 (SD 2·1) kg were used. Animals were randomised between three experimental groups and penned individually under 12 h light–dark conditions.

Abbreviations: AA, amino acid; ape, atom % excess; BAC, bacitracin diet; LS5, litter size of five pups; LS9, litter size of nine pups; NAB, no antibiotic diet; ppm, parts per million; VFA, volatile fatty acid.

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Diets

The basal diet was formulated (g/kg) with grass hay (400), wheat grain (200), soyabean meal (150), barley grain (130), sugarbeet pulp (100), sunflower-seed oil (5), ammonium sulfate (5) and a vitamin–mineral mix (10) with the following declared composition: Co (CoSO₄·7H₂O), 200 parts per million (ppm); Cu (CuSO₄·5H₂O), 3000 ppm; Fe (FeSO₄·H₂O), 20 000 ppm; Mn (MnO₂), 8000 ppm; Zn (ZnO), 30 000 ppm; Se (Na₂SeO₃), 30 ppm; I (KI), 500 ppm; vitamin A, 270 μkat (4 500 000 IU)/kg; vitamin D₃, 33 μkat (550 000 IU)/kg; vitamin E, 1100 ppm; vitamin B₁, 250 ppm; vitamin B₂, 1500 ppm; vitamin B₆, 100 ppm; vitamin B₁₂, 6000 ppm; vitamin K, 500 ppm; d-pantothenate, 5000 ppm; niacin, 12 500 ppm; choline chloride, 100 000 ppm. Its chemical composition (per kg fresh matter) was 91·25 g DM, and (per kg DM) 92·12 g organic matter, 19·06 g crude protein, 19·52 g acid-detergent fibre, 31·91 g neutral-detergent fibre, 4·81 g acid-detergent lignin and 2·67 g ether extract.

From 4d before predicted parturition (day 1 of the experimental period) the groups were fed one of three experimental diets as follows: the control group received a basal diet. This group was used mainly to obtain the background amino acid enrichments. The second group (no antibiotic diet; NAB) received the basal diet plus ammonium sulfate (5 g/kg), either unlabelled (day 1 to day 8) or labelled (10 atom % for 24 h to dislodge and isolate adherent bacteria) with [¹⁵N]lysine (AA) enrichments in milk, microbial and liver tissue were measured by GC-combustion-isotope ratio MS, as described by Belenguer et al. (3). AA concentrations were determined by HPLC using the Waters Pico-Tag method that involves pre-column derivatisation with phenylisothiocyanate (10).

Calculations

Bacteria are considered to form the entire microbial population in the rabbit caecum and throughout the text bacterial and microbial are both used to refer to the caecal microbial population in the rabbit. The contribution of microbial lysine (Mlys) to tissue lysine was estimated as described previously (3):

\[
\text{Mlys} = \frac{E_{\text{Tis}} - E_{\text{bac}}}{E_{\text{bac}} - E_{\text{bacC}}},
\]

where \( E_{\text{Tis}} \) and \( E_{\text{bac}} \) are [¹⁵N]lysine enrichments (atom %) in tissues and bacteria in animals fed the labelled diets while \( E_{\text{bacC}} \) are the corresponding enrichments from animals fed the unlabelled control diet.

Absorbed microbial lysine derived from caecotrophy (MlysA) was calculated as:

\[
M_{\text{lysA}} = \frac{M_{\text{lysCec}} \times D_{\text{lysA}}}{1 - M_{\text{lysCec}}},
\]

where \( M_{\text{lysCec}} \) and \( M_{\text{lysCec}} \) are the respective contributions of microbial lysine through the caecotrophy process and direct intestinal absorption to tissue lysine and \( D_{\text{lysA}} \) is the dietary supply of absorbed lysine, estimated from a true ileal digestibility of 0·80 (3). \( M_{\text{lysCec}} \) was assumed to be similar to the value estimated in growing rabbits (3 %), and \( M_{\text{lysCec}} \) was estimated by difference between \( M_{\text{lys}} \) and \( M_{\text{lysCec}} \).

Microbial intake (g DM/d) was calculated as follows:

\[
\text{Microbial intake} = \frac{M_{\text{lysA}}}{D_{\text{lysA}}},
\]

where \( D_{\text{lysA}} \) is the true digestibility of microbial lysine (0·897) (13) and \( D_{\text{lysA}} \) is the lysine concentration in caecal bacteria (g lysine/g DM).
Statistical analysis

Data were analysed by ANOVA as a $3 \times 2$ factorial design, with diet, litter size and their interaction as main effects. Residual plots were inspected for normality and homogeneity of variance, and where necessary the data were log-transformed (enrichments). When measurements over time were taken (food intake, body-weight changes and milk yield), data were analysed by residual maximum likelihood (REML), with animal as a random effect and period, diet, litter size and their interactions as fixed effects. Various covariance matrix structures, including autoregressive and unstructured matrices, were investigated for the within-animal stratum. It was found that assuming identical correlations between the periods in combination with different variances for each of the three time periods (so-called uniform correlation with heterogeneous variance) gave the best fit, based on comparison of deviances using Akaike’s information criterion.

For the AA enrichment data only, two diets were considered (BAC and NAB; Table 4) and they were analysed by ANOVA on log-transformed enrichments, for each amino acid individually. Animal was regarded as a random effect. Homogeneity of variances was investigated as follows. For the within-animal stratum, various covariance matrix structures were investigated, such as uniform (the ‘standard’ assumption), uniform with heterogeneous variance, and diagonal and unstructured covariance matrices, which were fitted using residual maximum likelihood. Models were compared using Akaike’s information criterion, and it was found that the uniform structure fitted the data best. As a consequence, the statistical analyses simplified to a split-plot ANOVA with animal stratum. Total VFA concentration was 46·2 mM, with acetic acid the most abundant VFA (C2; 71·6 %) followed by butyric (C4; 20·8 %) and propionic (C3, 7·5 %) acids. Although apparently total VFA concentration was lower with the antibiotic (NAB LS9 322 284 293 20·4; BAC LS5 313 330 319 28·0; Control LS5 322 267 322 2·3), these differences were not statistically significant.

Results

All animals remained in good health throughout the experimental period except one NAB animal (LS9) that was not able to adapt to the experimental diet and was removed from the study. Where no interaction was detected between litter size and antibiotic supplied, only main effects are presented in the tables.

Performance of lactating does

As lactation progressed, milk production increased ($P<0.001$) together with body-weight loss ($P=0.011$). Does gained weight in L1 (16·2 g/d), and started to lose weight in L2 (−5·2 g/d), but significant losses (−11·7 g/d) were detected during L3. No significant changes in food consumption were observed through lactation (Table 1).

Litter size, induced by cross-fostering, impacted on milk yield ($P=0.006$), but neither feed intake nor body weight was affected. No antibiotic effect was detected either on DM intake, body-weight change or milk yield.

Caecal parameters and amino acid concentration in bacteria, liver and milk

In lactating does, the caecal weight (282·2 (SE 6·99) g) represented 7 % of body weight and the pH of the caecal contents averaged 6·45. Neither was affected by experimental treatment. Total VFA concentration was 46·2 mM, with acetic acid the most abundant VFA (C2; 71·6 %) followed by butyric (C4; 20·8 %) and propionic (C3, 7·5 %) acids. Although apparently total VFA concentration was lower with the antibiotic (61·5 vs. 33·2 mM for NAB and BAC animals respectively).

Table 1. Effect of experimental diet (no antibiotic diet (NAB), bacitracin diet (BAC) or control diet), litter size and period of lactation on feed intake, body-weight changes and milk yield in lactating does (Mean values and standard errors of difference)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Litter size</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAB</td>
<td>LS9</td>
<td>370</td>
<td>338</td>
<td>289</td>
<td>22·7</td>
<td>−13·4</td>
<td>−15·5</td>
<td>176</td>
<td>203</td>
<td>216</td>
</tr>
<tr>
<td>NAB</td>
<td>LS5</td>
<td>322</td>
<td>264</td>
<td>293</td>
<td>13·2</td>
<td>−9·7</td>
<td>−5·5</td>
<td>135</td>
<td>149</td>
<td>197</td>
</tr>
<tr>
<td>BAC</td>
<td>LS9</td>
<td>313</td>
<td>330</td>
<td>319</td>
<td>28·0</td>
<td>−2·6</td>
<td>−14·7</td>
<td>138</td>
<td>178</td>
<td>213</td>
</tr>
<tr>
<td>BAC</td>
<td>LS5</td>
<td>309</td>
<td>320</td>
<td>281</td>
<td>14·7</td>
<td>7·3</td>
<td>−20·1</td>
<td>128</td>
<td>146</td>
<td>172</td>
</tr>
<tr>
<td>Control</td>
<td>LS9</td>
<td>353</td>
<td>314</td>
<td>298</td>
<td>18·5</td>
<td>−11·9</td>
<td>−13·5</td>
<td>173</td>
<td>195</td>
<td>209</td>
</tr>
<tr>
<td>Control</td>
<td>LS5</td>
<td>322</td>
<td>267</td>
<td>322</td>
<td>2·3</td>
<td>−0·6</td>
<td>−1·2</td>
<td>136</td>
<td>142</td>
<td>194</td>
</tr>
<tr>
<td><strong>SED</strong></td>
<td></td>
<td>36·2</td>
<td>42·3</td>
<td>39·9</td>
<td>20·4</td>
<td>7·96</td>
<td>18·5</td>
<td>25·9</td>
<td>24·6</td>
<td>39·5</td>
</tr>
</tbody>
</table>

**Statistical significance**

- Period: $P<0·001$
- Diet: $P=0·011$
- Litter size: $P<0·001$

* Data were analysed by residual maximum likelihood (REML), assuming uniform correlation with heterogeneous variance for the three time periods. The two- and three-way interactions were non-significant ($P>0·10$).

LS9, litter size of nine pups; LS5, litter size of five pups; L1, days 1 to 8 of lactation; L2, days 9 to 17 of lactation; L3, days 18 to 25 of lactation.
Protein recycling in lactating does

no significant differences were observed (Table 2), and VFA proportions were also unaltered.

Total analysed AA concentration (mg/g DM; Table 3) was highest in liver (557 (SE 32.8)), followed by bacteria (468.4 (SE 11.9)) and diet (140 (SE 6.6)), of which lysine represented 7.03, 6.49 and 4.83 %, respectively.

15N enrichment in caecal ammonia and urine urea

In the NAB group, average 15N abundance in urine urea (0.87 (SE 0.015) atom %) was greater (P<0.05) than in microbial-N (0.61 (SE 0.076) atom %), and the latter was similar to the caecal ammonia enrichment (0.63 atom %). Assuming that urine and plasma urea have the same 15N enrichment and based on a natural abundance of 0.366 atom %, the maximum contribution of plasma urea-N to caecum bacterial-N was 0.48 ((0.610 - 0.366)/(0.870 - 0.366)), with the remainder derived from dietary sources. If, however, caecal ammonia were the precursor then, by similar reasoning, this would account for >0.90 of microbial-N, with only a small contribution required from feed residue in the large intestine. The ammonia option is probably closer to the real situation.

15N enrichment in amino acids

No effects of diet or litter size were detected on [15N]amino acid enrichment and therefore mean 15N enrichment values for each of the thirteen AA monitored in bacteria, liver and milk are presented in Table 4. Differences in lysine enrichment between the various treatments (litter size and NAB v. BAC) were small (2 % for bacteria, 6 % for liver and 5 % for milk, respectively). Enrichments (atom % excess; ape) in threonine were more variable but the numerical differences between diet (0.069 for NAB and 0.077 for BAC) and litter size (0.071 for LS5 and 0.075 for LS9) were not statistically significant. The 15N enrichments differed (P<0.05) between individual AA, both between bacteria, liver and milk and within bacteria. In the microbial protein, threonine had the greatest enrichment (0.31 ape). Most of the other AA had similar enrichments (from 0.279 to 0.231 ape) in the order proline, glutamate, isoleucine, aspartate, valine, leucine, phenylalanine serine and lysine and these were all higher (P<0.04) than proline and glycine (0.068 and 0.197 ape, respectively). All AA showed a greater enrichment in microbes than in either milk or liver (P<0.001). With the exception of phenylalanine (P=0.96), enrichments were greater in milk than in liver (P<0.001 for most amino acids except for proline P=0.017, tyrosine P=0.020, and threonine P=0.035).

Caecotrophy contribution

Microbial lysine contribution to tissue lysine was estimated based on a natural abundance of 0.366 atom %, with the remaining derived from dietary sources. If, however, caecal ammonia were the precursor then, by similar reasoning, this would account for >0.90 of microbial-N, with only a small contribution required from feed residue in the large intestine. The ammonia option is probably closer to the real situation.

Discussion

15N lysine approach

Caecotrophy is a crucial mechanism to enhance protein supply in rabbits. This process has been quantified for growing rabbits(3,5), but few studies have investigated the contribution in lactating does(7). Furthermore, conventional methodology, based on a wooden collar, is unsuitable for lactating does. In the present study, an alternative non-invasive methodology, based on incorporation of inorganic 15N into microbial lysine and validated already against the conventional procedure in growing rabbits(3), has been employed.

Microbial lysine used for body protein synthesis can arise either from direct absorption from the small intestine, as occurs in pigs(1), or from caecotrophy. In a previous study with collared growing rabbits, Belenguer et al.(3) estimated that <3 % of microbial lysine was incorporated from direct gut absorption and a similar value is assumed for the present study.

In growing animals the microbial contribution was estimated using liver protein because this has a high rate of protein turnover and 15N enrichment may reach plateau in a shorter time(3). Nevertheless, certain hepatic proteins, such as those involved in cell structure, may have low rates of

Table 2. Effect of experimental diet (no antibiotic diet (NAB), bacitracin diet (BAC) or control diet) and litter size on caecum weight, pH and volatile fatty acid (VFA) concentrations and proportions of acetic, propionic and butyric acids in lactating does

<table>
<thead>
<tr>
<th>Diet</th>
<th>Litter size</th>
<th>Weight (g)</th>
<th>pH</th>
<th>Total VFA (mM)</th>
<th>Acetic acid (%)</th>
<th>Propionic acid (%)</th>
<th>Butyric acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAB</td>
<td>LS9</td>
<td>279</td>
<td>6.09</td>
<td>69.3</td>
<td>68.3</td>
<td>6.9</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>LS5</td>
<td>264</td>
<td>6.48</td>
<td>53.8</td>
<td>74.8</td>
<td>6.7</td>
<td>18.5</td>
</tr>
<tr>
<td>BAC</td>
<td>LS9</td>
<td>300</td>
<td>6.61</td>
<td>34.5</td>
<td>71.5</td>
<td>7.2</td>
<td>21.3</td>
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<td>LS5</td>
<td>289</td>
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<td>32.0</td>
<td>69.9</td>
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<tr>
<td>Control</td>
<td>LS9</td>
<td>289</td>
<td>6.49</td>
<td>45.7</td>
<td>74.4</td>
<td>7.5</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>LS5</td>
<td>272</td>
<td>6.30</td>
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<td>70.7</td>
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<td>3.61</td>
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<table>
<thead>
<tr>
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<tbody>
<tr>
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<tr>
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<tr>
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<td>0.78</td>
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<td>0.52</td>
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<tr>
<td>0.78</td>
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<tr>
<td>LS</td>
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<tr>
<td>0.35</td>
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<td>0.86</td>
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<tr>
<td>0.27</td>
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<td>0.47</td>
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</tbody>
</table>

LS9, litter size of nine pups; LS5, litter size of five pups.

* Data were analysed by two-way ANOVA. The interaction between diet and litter size was not significant (P>0.10).
Enrichment of amino acid-nitrogen in bacteria and tissues

There are various sources of \(\text{N}\) for microbes in the caecum, and differences in amino acid enrichments in microbial protein reflect differential inflows from labelled ammonia, body proteins and undigested dietary residues. The contributions of these various \(\text{N}\) sources vary between AA, as already demonstrated for ruminants\(^{17,18}\). The AA enrichment patterns in caecal bacteria of the lactating does are not dissimilar to previous values reported in fattening rabbits\(^{15}\). In general, the high enrichment of glutamate and aspartate would confirm the central role played by glutamate as an intermediate in \(\text{N}\) transfer between AA\(^{17}\). The lowest enrichments corresponded to glycine and proline and such values agree with previous observations in ruminants\(^{17,19}\). These low enrichments are unlikely to be due to high rates of intestinal supply of unlabelled proline but it is known that l-proline is a strong allosteric inhibitor of proline synthesis (through glutamate quinase). Therefore, moderate supply of preformed proline, either from undigested dietary protein or tissue sources, may limit synthesis \textit{de novo} and indeed Atasoglu et al.\(^{19}\) indicated that microbial biosynthesis of proline is markedly reduced if proline is supplied directly.

Tissue AA enrichment depends on the amounts and enrichments of the two ‘exogenous’ sources, bacteria and food, plus metabolism within the animal. For AA that undergo extensive transamination, for example, glutamate, aspartate and alanine of non-essential AA and valine, leucine and isoleucine of the essential AA, such actions will obscure the contribution of bacterial amino acid-carbon to tissue protein. A better index is given by essential AA that do not undergo transamination, such as lysine and threonine. Threonine was the most enriched AA in bacterial extracts, but had the lowest contribution from microbial sources. Relative to lysine, the ratio tissue:bacterial \(\text{N}\) enrichment in threonine was 2-fold lower. Threonine is an important component of mucins, and a direct utilisation of substantial quantity of enteral sources (including recycled bacterial protein) by gut tissue for mucin synthesis may limit the amount available for peripheral tissues\(^{20,21}\) and would delay the equilibrium in threonine enrichment within plasma and tissues.

**Microbial contribution to amino acid requirements**

The aim of the present experiment was to study the contribution of microbes to amino acid requirements during lactation and how this is affected by different levels of intake, induced by altering the litter size, and the inclusion of dietary bacitracin. The addition of bacitracin in the diet, however, did not affect microbial contribution, and that fits with recent observations that this antibiotic does not alter bacterial biodiversity in lactating rabbits\(^{22}\).

In growing rabbits, caecotrophy can contribute up to 0.38 of total protein intake\(^{23}\). Higher values may be observed with adults at maintenance or on a protein-free diet\(^{22}\). The heaviest protein demand, however, arises during lactation. During late lactation the microbial contribution to tissue lysine was
Protein recycling in lactating does

Table 5. Effect of experimental diet (no antibiotic diet (NAB) or bacitracin diet (BAC)) and litter size on dietary and microbial contribution through caecotrophy ($M_{\text{lyz}}(\text{Cec})$) to milk lysine, absorption of dietary ($D_{\text{lyzA}}$) and microbial lysine through caecotrophy ($M_{\text{lyzA}}$) and microbial intake in lactating does fed on a $^{15}\text{NH}_4\text{SO}_4$-supplemented diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Litter size</th>
<th>Contribution to tissue lysine</th>
<th>Lysine absorption (g/d)</th>
<th>Microbial intake (g DM/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$M_{\text{lyz}}(\text{Cec})$</td>
<td>$D_{\text{lyzA}}$</td>
<td>$M_{\text{lyzA}}$</td>
</tr>
<tr>
<td>NAB</td>
<td>LS5</td>
<td>0.765</td>
<td>0.225</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>LS9</td>
<td>0.773</td>
<td>0.217</td>
<td>1.65</td>
</tr>
<tr>
<td>BAC</td>
<td>LS5</td>
<td>0.752</td>
<td>0.238</td>
<td>1.35</td>
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<td></td>
<td>LS9</td>
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</tr>
<tr>
<td>Statistical significance*</td>
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<td></td>
</tr>
<tr>
<td>Diet</td>
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<td>0.12</td>
<td>0.02</td>
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<tr>
<td>Litter size</td>
<td>0.88</td>
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</tr>
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</table>

* Data were analysed by two-way ANOVA. The interaction was not significant ($P > 0.10$).

0.23 (se 0.035) based on milk protein, similar to liver-derived values in lactating does (0.18 (se 0.048))24 using ion-exchange chromatography and growing rabbits (0.23 (se 0.057))25 fed different sources of carbohydrate. The voluntary intake of lactating females is 30% greater than in growing rabbits25 and 3-fold higher than for adult animals26. Such differences would impact on the caecal environment and microbial yield. Indeed, microbial lysine absorption was four times greater in these lactating does than for growing rabbits (0.12 g/d)3. This represents more than 25% of the apparent digestible lysine requirement of lactating does (1.8 g/d, assuming a mean value of 300 g DM intake/d20).

Therefore, although intake of soft faeces during lactation was much greater than observed in growing rabbits, the relative contribution of caecotrophes to total N supply was similar because the lactating animals ate proportionally more. As with ruminants, amino acid requirements of the rabbit are met by both dietary and microbial protein but one advantage of ingestion of microbial protein (or caecotrophes) is the higher content of the limiting AA, lysine, methionine and histidine compared with plant protein29. AA requirements for lactating does remain to be established30 but the high concentration of lysine and methionine in both the whole body and milk (383 and 77 v. 451 and 150 mg/g N, for lysine and methionine, respectively30) confirms why it is important for the doe to improve microbial protein intake. Comparison of these data with those in growing rabbits3 might suggest, however, that caecotrophes ingestion or production may have limits. If this is the case, then is caecotrophy restricted by a physical limitation of ingestion or because of an unbalanced nutrient supply? Although four-fold more caecal-derived lysine was ingested during lactation than growth this was still not enough to support both milk production and maintain the nutritional requirements of the mother, as these lose weight. Partly this was due to intake either being maintained or even reduced as lactation progressed but while milk output increased. During this period, demands for both protein and energy are increased but the doe has to balance intake of the diet, with higher energy but lower protein quality, against caecal material that offers good-quality protein but relatively little energy. Such a balance may not be fixed, however, and supply of a better-quality (or more energy-dense) diet may alter the amount of recycled bacterial protein and permit the mother to maintain both her lean and adipose tissue stores. This hypothesis needs to be tested.

The microbial lysine incorporation method is non-invasive and probably the most suitable to estimate microbial protein intake in lactating does because it does not alter animal metabolism or behaviour. Despite the importance of the caecotrophy process to doe nutrition, N recycling was affected neither by ingestion of antimicrobial substances that inhibit microbial yield nor through changing nutrient demand by manipulating litter size. Why the caecotrophic response remains constant even with these altered demands and pressures has yet to be answered.

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