Influence of maternal feed rationing on metabolic and contractile properties of *Longissimus lumborum* muscle fibres in the rabbit offspring

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Abstract

Thirty hybrid female rabbits of 15 weeks of age were divided into three groups and fed for 8 weeks (until the first parturition) on one of the three following diets: “C diet” (DE = 11.71 MJ/kg DM) fed ad libitum; “R diet” was the C diet fed at 80% of ad libitum, “F diet” rich in fibre (24.6% vs 18.7% for C diet; DE = 9.77 MJ/kg DM) fed ad libitum. Afterwards, all the does received the C diet ad libitum. One pup per litter was slaughtered at birth, one at 35 d (weaning) and one at 81 d of age. At the second parturition, another pup per litter was slaughtered at birth. Ten minutes post mortem the *Longissimus lumborum* (LL) muscle was sampled and the mean cross-sectional area, compactness index (CI) and sphericity of the fibres of the new-born rabbits were determined; on rabbits of 35 and 81 d of age the fibre type distribution (βR, αR or αW) was also measured. The two maternal rationed-diets significantly increased the CI of LL fibres of new-born rabbits of the first kindling, compared to the C diet. On weaning rabbits the greatest effect of maternal feed rationing was observed on the percentage of αW fibres (P < 0.01), which was the highest for the F diet (85.2%), intermediate for the C diet (78.0%) and the lowest for the R diet (71.8%). At 81 d of age, no effect of maternal dietary treatment was found on offspring.

Keywords: Rabbit; Doe; Feed rationing; Muscle; Fibre type

1. Introduction

The young nulliparous rabbit does fed ad libitum with diets of high energy level often show parturition problems, with the subsequent reduction of the number of new-born rabbits, linked to excessive fatness (Fortun-Lamothe & Lebas, 1996). The administration of energy-rich diets during lactation increased the milk output, but was not able to reduce the energy deficit normally induced by pregnancy, or by the simultaneous lactation and pregnancy, because the doe’s stomach volumetric capacity is limited and her intake of food cannot meet the requirements. In primiparous does, which are still growing, the stomach capacity is not enough to cover the doe’s requirements so, if pregnancy and lactation overlap, the energy deficit increases strongly.

In order to reduce the excessive fatness of young rabbit does, restricted feeding is frequently applied. A dietary energy restriction balanced with an increase of fibre content administered to young female rabbits until the first parturition, could increase the stomach and caecum volumetric capacity and, consequently, increase the energy intake during the following lactation, reducing the negative energy balance. Research on the effect of restricted feeding on reproductive performance of rabbit does is widely performed.
The “F” diet rich in young females (DE fed on one of the three following diets: the “C” diet for females were divided at random into three groups and weeks. After an adaptation period of 4 d the young individually housed in commercial cages at the age of 15 2.1. Animals, housing and experimental diets

2. Materials and methods

2.2. Slaughtering and sampling

The three pups identified per doe belonging to the first offspring were slaughtered at birth, at 35d of age (weaning) and at 81 d of age, respectively. After the slaughter at birth, the litter size per doe was seven until weaning. The pup identified at the second parturition was slaughtered at birth. Ten minutes post mortem the Longissimus lumborum (LL) muscle was sampled between the third and the seventh lumbar vertebra and then underwent the histochemical treatment (freezing in isopentane cooled by liquid nitrogen). Samples were then stored at \(-80^\circ\text{C}\) until analysis.

2.3. Histochemical analysis of the Longissimus lumborum muscle

Six serial cross-sections (10\(\mu\text{m}\) thick) from each LL sample, belonging to rabbits of 1, 35 and 81 d of age were obtained with a cryostat at \(-20^\circ\text{C}\). One was stained with azorubine (reference staining); four were processed according to the myofibrillar ATPase after successive preincubations in acid and alkaline buffers (Guth & Samaha, 1970) and one was stained for succinic dehydrogenase (SDH) activity (Nachlas, Tsou, De Souza, Chang, & Seligman, 1957). For the acid preincubation, three pH values ranging from 4.15 to 4.30, were tested on 3 sections and the one that gave the best fibre typing contrast was retained. The fourth one was used for alkaline preincubation (pH 10.4). Myofibrillar ATPase and SDH stain intensities were estimated for each individual cell and used for fibre classification according to Ashmore and Doerr (1971) as \(\beta R\), \(\alpha R\) or \(\alpha W\) by combination of metabolic (R, red or W, white) and contractile (\(\alpha\), fast-twitch or \(\beta\), slow-twitch) characteristics. Percentage, mean CSA (\(\mu\text{m}^2\)), compactness index (CI) (perimeter\(^2\)/area) and sphericity (SPH) (\(d/D\) = smallest diameter/biggest diameter) of each fibre type were determined on approximately 300 fibres in three random fields for each muscle with a computerised image analysis system (Buche, 1990). Fibres of the new-born rabbit’s LL muscle were all identified as \(\alpha R\) type, so, only histomorphological characteristics were determined on azorubine-stained fibres. The software Racine was used on a Unix workstation equipped with a graphics card (Imaging Technology FG100).

2.4. Statistical analysis

Variance analysis was performed using the GLM procedure of the SAS program (SAS Institute, 1990), by including diet as fixed effect.
3. Results and discussion

As the litter weight and size at birth did not differ significantly among the experimental groups, it can be considered that the nutrient supply to pups within each litter was similar (Saintonge & Rosso, 1981).

At birth all the fibres of LL muscle were typed as $\alpha R$. Table 1 shows the effect of the maternal feed rationing on the LL fibre CSA, CI and SPH belonging to 1-day-old rabbits of first and second kindling. Offspring coming from the first kindling did not show significant differences either for LL fibre CSA or for LL fibre SPH, linked to maternal dietary treatment. On the contrary, the maternal feed rationing, applied as qualitative restriction (diet F) or quantitative restriction (diet R) significantly increased the CI, compared with the C diet (1.84 vs 1.74, $P < 0.01$). Nevertheless, the CI values, which indicate fibre deformation induced by pathological states, is considered acceptable in the range of variation found in the present work. The histomorphological characteristics of LL fibres of the second kindling were unaffected by maternal feed rationing.

At weaning, the young rabbits belonging to the first kindling exhibited an effect of maternal feed rationing on fibre type distribution (Table 2). The R diet, compared with F and C diets, significantly increased the percentage of $\beta R$ fibres (10.2% vs 4.2% vs 4.2%, respectively; $P < 0.05$). On the contrary, F, compared with C and R diets, significantly reduced the percentage of $\alpha R$ fibres (10.5% vs 17.8% vs 18.8%, respectively; $P < 0.05$). The greatest effect of maternal feed rationing was observed on the percentage of $\alpha W$ fibres ($P < 0.01$), resulting in a higher percentage in animals on the F diet (85.2%), an intermediate level for those on the C diet (78.0%) and the lowest percentage in those on the R diet (71.8%). This reduction of the proportion of $\alpha W$ fibres of the offspring’s LL muscle, induced by quantitative feed rationing of the does, might be explained by a reduction in the number of secondary fibres which form, mainly the fast-glycolytic ones (Lefaucheur & Gerrard, 1998). Consequently, the reduction in type $\alpha W$ fibre proportion may decrease of the glycolytic metabolism in the LL muscle. A significant increase in the proportion of $\beta R$ fibres in the red Rhomboideus muscle was also observed in pigs feed rationed at 50% of the ad libitum intake at an early stage of growth (Harrison, Rowleson, & Dauncey, 1996). According to these authors, a selective increase in the proportion of $\beta R$ fibres during a period of reduced energy availability would be physiologically relevant as a way to spare energy.

Gondret, Fortun-Lamothe, and Bonneau (1997) have demonstrated that nutrient deficiency induced by concurrent gestation and lactation, if compared with only gestation, delayed the myofibrillar maturation rate of

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**Table 1**

<table>
<thead>
<tr>
<th>Diets</th>
<th>First kindling</th>
<th>Second kindling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Rabbits (n)</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Liveweight (g)</td>
<td>58.0</td>
<td>63.9</td>
</tr>
<tr>
<td>Fibre cross-sectional area ($\mu m^2$)</td>
<td>46.7</td>
<td>39.8</td>
</tr>
<tr>
<td>Compactness index ($\frac{\text{perimeter}^2}{\text{area}}$)</td>
<td>1.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sphericity ($d/D$)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Within a row, means with different superscripts (a, b) are significantly different.

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**Table 2**

<table>
<thead>
<tr>
<th>Diets</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>F</td>
</tr>
<tr>
<td>Rabbits (n)</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Liveweight at weaning (g)</td>
<td>759</td>
<td>675</td>
</tr>
<tr>
<td>Milk intake (g/d)</td>
<td>215.6</td>
<td>216.1</td>
</tr>
</tbody>
</table>

Fibre type distribution (%)

- $\beta R$: 4.2<sup>a</sup> vs 4.2<sup>a</sup> vs 10.2<sup>b</sup> * 4.4
- $\alpha R$: 17.8<sup>b</sup> vs 10.5<sup>b</sup> vs 18.0<sup>b</sup> * 5.7
- $\alpha W$: 78.0<sup>b</sup> vs 85.2<sup>b</sup> vs 71.8<sup>b</sup> ** 6.9

Fibre cross-sectional area ($\mu m^2$)

- $\beta R$: 31.1 vs 37.6 vs 348 | NS | 103 |    |    |
- $\alpha R$: 300 | 318 | 382 | NS | 135 |    |    |
- $\alpha W$: 498 | 494 | 608 | NS | 227 |    |    |

Compactness index ($\frac{\text{perimeter}^2}{\text{area}}$)

- $\beta R$: 2.10 vs 2.24 vs 2.10 | NS | 0.23 |    |    |
- $\alpha R$: 2.11 vs 2.17 vs 2.17 | NS | 0.19 |    |    |
- $\alpha W$: 2.15 vs 2.21 vs 2.16 | NS | 0.16 |    |    |

Sphericity ($d/D$)<sup>a</sup>

- $\beta R$: 0.59 vs 0.53 vs 0.61 | NS | 0.07 |    |    |
- $\alpha R$: 0.62 vs 0.62 vs 0.58 | NS | 0.09 |    |    |
- $\alpha W$: 0.59 vs 0.57 vs 0.64 | NS | 0.05 |    |    |

Within a row, means with different superscripts (a, b) are significantly different.

- $d$ = smallest diameter; $D$ = biggest diameter.
- NS, not significant: $P > 0.05$.
- SEM is the standard error of the least squares means.
- * $P < 0.05$.
- ** $P < 0.01$. 
offspring determined at 29 d of age, but at 70 d of age no effect was found on fibre type proportion. Analogous to this, in the present work the probable nutrient deficiency of does induced by feed restriction, which overlapped the concurrent gestation and lactation physiological states, delayed the fibre differentiation of the offspring’s LL muscle. The delay was detectable until the weaning age of 35 d.

The delay in the myofibrillar maturation rate could derive either from a maternal effect during the intra-uterine life or from the successive impaired doe’s milk production.

In the first case, a deficit in maternal feeding status could produce a pre-natal under nutrition and, as a consequence, could reduce the total number of fibres and modify the muscle energy metabolism of the offspring, as observed in pigs by Vigneron, Bacou, Nouguès, and Lefauuche (1983). Also according to Rehfeldt, Stickland, Fiedler, and Wegner (1999) the prenatal period of muscle development is more sensitive to nutritional deficiencies in reducing the number of fibres in pigs, guinea pigs and rats. However, a recent study on rats (Bayol, Jones, Goldspink, & Stickland, 2004) did not find variation of the total number of fibres in the Semitendinosus muscle and muscle CSA of 21-days-old pups when severe gestational undernutrition (40% of ad libitum) was applied. In the present work, the total number of fibre was not assessed, but the similar liveweight at birth of the young, derived from control and rationed does, seem to suggest only a slight effect on foetal undernutrition (Table 1).

In the second case, the young born to does of group R could have been undernourished also during the suckling period because of the lower milk production (209.6 vs 215.9 g/d) of the other two groups; Table 2): This double undernutrition could have amplified or maintained the previous dietary maternal effect of a decrease in glycolytic metabolism.

At weaning, the histomorphological characteristics of LL muscle were not significantly influenced by the maternal dietary treatment; however the fibre CSA of αR and αW fibre types seemed to be higher in weaned rabbits of group R, when compared to the other two dietary treatments (Table 2). The same trend was found for fibre CSA of new-born rabbits.

At 81 d of age, no effect of maternal dietary treatment was found in the offspring, either on fibre type distribution or on fibre histomorphological characteristics (Table 3). The same diet administered to all rabbits from weaning to commercial slaughter age nullified the effect of the maternal diet. However, rabbits of the R feeding group showed a slight compensatory growth and also seemed to compensate for the low glycolytic metabolism induced by the maternal quantitative feeding restriction: when compared with C and F groups, the percentage of αW fibres of 81-day-old rabbits strongly increased after weaning (from 71.8% at weaning to 86.3% at 81 d) while the percentage of βR fibres strongly decreased (from 10.2 at weaning to 1.7% at 81 d).

**Acknowledgement**

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**References**

Fortun-Lamothe, L., & Lebas, F. (1996). Effects of dietary energy level and source on foetal development and energy balance in concur-


