




# Peer Community In Animal Science

## Managing the feeding of rabbits to improve metabolic efficiency

**Giuseppe Conte** based on peer reviews by **Marion Boutinaud**, **Davi Savietto**  and 1 anonymous reviewer

Cathy Hue-Beauvais, Karine Bebin, Raphael Robert, Delphine Gardan-Salmon, Mickael Maupin, Nicolas Brun, Etienne Aujean, Florence Jaffrezic, Steve Simon, Madia Charlier, Fabienne Le Provost (2022) Impact of pre-breeding feeding practices on rabbit mammary gland development at mid-pregnancy. Missing preprint\_server, ver. Missing article\_version, peer-reviewed and recommended by Peer Community in Animal Science.

<https://doi.org/10.1101/2022.01.17.476562>

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A correct execution of feeding plan for growing rabbit decreases the possibility of post-weaning digestive disorders, thus enhancing the feed efficiency in the animals. However, a limitation of feed daily quantity is required between 10 weeks of age and the first artificial insemination. This limitation causes energy deficiency with a consequent reduction in fertility. Beauvais et al. (2022) studied the impact of feed restriction strategies in female rabbits. Four feed restriction strategies were applied in two distinct periods (post-weaning and puberty) and evaluated by different physiological parameters (growth rate, metabolic profiles, reproductive parameters and mammary gland development). In the first part of the paper, the authors evaluated the association between weight slopes and feeding strategies in the late post-weaning and peripartum period in the four groups. As revealed by the authors, a significant difference was observed during the late post-weaning period, which remained significant between the pubertal and fattening phases. Probably these differences are related to the restriction feeding pattern. The results indicated that restrictive feeding changes in the first step of post-weaning period is associated with a significant difference in body weight. This difference occurs from the third week of diet, highlighting the high sensitivity of growing rabbit to nutrition during the post-weaning period.

In the second part of the paper, the authors evaluated the expression of genes involved in the lipid metabolism. During the mid-pregnancy, was revealed a significant higher expression of lipogenic genes, which may be considered as useful markers for the mammary epithelial development in less restrictive strategies during early life period.

The results proposed by Beauvais et al. (2022) enlighten the important role played by the feeding conditions of young female rabbits in the early life rearing. In particular, this activity provides specific recommendations for optimizing lactation and thus preventing neonatal mortality of the offspring. Moreover, the authors provide indications about the effect of feeding strategies on the mammary development and gene expression with absolute consequences on the development of offspring. Mammary lipid metabolism affects the milk profile and therefore the growth performance of the young animals.

### **References:**

Hue-Beauvais C, Bebin K, Robert R, Gardan-Salmon D, Maupin M, Brun N, Aujean E, Jaffrezic F, Simon S, Charlier M, Le Provost F (2022). Impact of pre-breeding feeding practices on rabbit mammary gland development at mid-pregnancy. *bioRxiv*, 2022.01.17.476562, ver. 3 peer-reviewed and recommended by Peer Community in Animal Science. <https://doi.org/10.1101/2022.01.17.476562>

## **Reviews**

### **Evaluation round #1**

DOI or URL of the preprint: <https://biorxiv.org/cgi/content/short/2022.01.17.476562v1>

### **Authors' reply, 16 May 2022**

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### **Decision by [Giuseppe Conte](#), posted 25 March 2022**

#### **Major revision**

Dear authors,

experts have read and evaluated your work. Everyone agrees that the work is interesting. However, some changes are needed in order to improve the quality of the manuscript.

I invite you to follow the suggestions of the reviewers.

Giuseppe Conte

### **Reviewed by [Marion Boutinaud](#), 23 March 2022**

The paper presents a study about the effect of pre-breeding feeding practices on rabbit mammary gland development. The rabbits were exposed to two feed restriction periods during their development. I found several merits in this pre-print. The study presents several analyses conducted on the mammary tissue (gene expression and histological analyses). The results shown provide evidence that the feeding strategies influence the mammary development and gene expression. The introduction clearly explains the motivation for the study. The research hypothesis is clearly presented. The introduction builds on relevant recent and past research performed in the field. However, I have several minor comments on the material and method parts. My major concern is about the statistical analyses. I consider that the statistical analyses should be completely revised. The new method suggested will probably give the same types of results as the ones already observed. However, a global model will be more appropriate. In this model, the authors could include the analyses of the effects of each type of restriction (at post weaning and during puberty) and the interaction of both treatments using an ANOVA. And finally, the abstract and the conclusion deserve to be more precise.

## Abstract

L29-30 the sentence is not clear to me, what is the problem with the common practices during rearing in commercial rabbit production

L33 the authors analyzed 4 strategies with two periods of restriction. Please modify the text

Please check the English language for example L39-40-43 please use the past tense: have/ had vary / varied, have impacted /impacted; and L336 "compared with" instead of" compared to "...

L39 what kind of reproductive parameters were measured in this paper?

L40-L41 and L43 be more specific about the results obtained. What were the effects?

L42 When did you collect the mammary tissue samples? At what period the gene expression was studied? This should be indicated in the abstract.

L43 did you perform the analyses in the mammary epithelial cells or in the mammary tissue ? Did you isolate mammary epithelial cells?

Please indicate the p-value when the effect is significant L41-46

L45 what does mean regarding further lactation?

L479 front my point of view, the conclusion is not clear enough about what is the best strategy for the mammary gland development.

L60-61 The expression "reared the same way as fattening animals" is not clear to me

L64 kit survival. Please remove "s" to "kits"

L91-L93 as previously written in the introduction part, the effect of restricted feeding strategy is known in gilts and goats. Please indicate here the rabbit specie

L111 the number for the authorization from the French Ministry of Agriculture should be mentioned here.

L118-120 Please clarify the amount of feed given to the does in the SR Group. According to Fig 1 it depends on the live weight. This information is lacking in the text.

L120 I may did not well understand but I suggest to change the text to "from 9 to 11" instead of "12".

L128 the method of sacrifice should be described here.

L129 why a fasting has been performed before the sacrifice? This has been explained later in the text, but should be clarified in that section.

L129 what is a hydrous fasting? The remove of food and water? of only water or only food?

L133 the method of sacrifice should be mentioned in the previous paragraph.

L136-139 did you measure the weight of the excised mammary tissue? Maybe this information could give an idea of the mammary development?

L147 I did not well understand the number of sample per rabbit. In the previous paragraph, it is mentioned that only left inguinal glands were excised.

L148 the percentage of areas

L166 only one gene was used as a reference gene. Nowadays at least 2 reference genes are asked. Did you check if the expression of Tbp gene was stable according with your different groups of animals?

L175-179 The statistical analyze should be completely revised. Since you performed two strategies of feed restriction one during post weaning and one during Puberty, you can consider these two strategies as two treatments and you could analyze the effects of both treatments and the interaction of both treatments using an ANOVA. You also need to check if your data can fit or not with this type of analyze (the linearity of the residus and the homogeneity of the variance of the residus).

Here you wrote that you performed either student's t-test of Mann Whitney U test. The authors should indicate what tests have been considered for each variable. When you considered your data according to

the effect of time (for live weight), you also should consider the “rabbit effect” as either a repeated effect or a random effect, and the interaction between the time and the two treatments

L193-220 each time a numerical results (L193, 199...) is shown please provide the sem.

L193-212 When the effect is significant or not please provide the P-values (L200, 202, 203, 204, 206, 208, 211, 213, 219, 220...L237, L239, L240, L242, L250, L251)

L193-L267. When the statistical analyzes will be revised the data should be presented according to the new statistical analyze showing the p-value for the effect of the feed restriction at post weaning, the effect of feed restriction at puberty and the effect of the interaction of both

Figure 1, 3, 4, 6 and 7 please add a space before and after = (n = 10)

Figure 2-6 please indicate each treatments in the legends as for Figure 1

Figure 2 how did you measure the ingestion? This should be indicated in the material and method part

Figure 2 These data should be analyzed including a time effect

Figure 1 The average daily food intake given in the table A should be given with the mean  $\pm$ SEM. You could test the effect of each treatment on these data and give the p-value of the effect of the feed restriction at post weaning, the one for feed restriction at puberty and the one for the interaction of both. These P values should also be given in the legends of Figure 3, 4, 5 and 6

Figure 1 legend. Please add the species “Each group of rabbits”

Figure 1 table B “Puberty” instead of “Pubertal”

Figure 4 a table is probably more appropriate since no effect seems to be observed. A table with the means mean  $\pm$ SEM and the p-value for the different effects-

Figure 5 Please modify the title of the (B). My suggestion “Relative quantification of areas occupied by the different types of mammary tissue”

Figure 5 please add % of area in the Y-axis legend

L295 please add as expected before consequently

L311-318 : the blood metabolic parameters have been measured at euthanasia after 12 hours of fasting. In the discussion, the authors should maybe suggest that these parameters were not measured at the appropriate time to observe the effect of feed restriction. The blood sample collection should have been done during the period of feed restriction and not at D14 of pregnancy when the both feed restriction periods are over. So I don't agree with the last sentence of the paragraph L317. I wonder if there is any interest in showing these results.

L325 () ?

L334 since the area occupied by fat and epithelial tissue were considered together you can't really write that it is due to a combined decrease in fat and epithelial tissues. You don't know if it is due to fat epithelial or both. Please change this.

L335-337 in the result part and in figure 5 it is not mentioned that the luminal part is higher in the most restricted animals than in any other groups of animals. Please revise this sentence

L338-340 The authors seemed to observe an interaction between your feed restriction treatments on the % of area occupied for connective tissue. Could you give some hypothesis using what you know about the period of mammary development (epithelial versus connective tissue during post weaning or pubertal period).

L354-355 is the reference Takeuchi et al.2001 really useful here? In Takeuchi et al. they studied the effect of different types of lipid supplementation on the liver whereas the authors here studied the effect of feed restriction on the mammary tissue. So there is no link between both studies

L368 what tend to be increased mean here? With the P-value this will help to understand. This information should be given in the result part and not in the discussion.

L385-L396 this part seems to be disconnected of the results obtained in the study. So do you really need to add this paragraph? The link with the objectives of the study in not clear.

L397 to conclude what is the most critical period for feed restriction? Using a ANOVA model you could see which period is more critical for mammary development.

L402 your conclusion is that a severe restriction could adversely affect mammary tissue and not health. Please remove health here. Please explain which phase is the most critical and what are the conclusion of your study.

**Reviewed by [Davi Savietto](#) , 18 March 2022**

[Download the review](#)

**Reviewed by anonymous reviewer 1, 25 March 2022**

The authors have conducted a straightforward experiment. The results are correctly described and explained. In general, the manuscript is well written, easy to read and understand. I think it is a poorly explored theme in the rabbit but very important and interesting

The figure 1A is very helpful to understand the experimental plan, on the contrary the figure 2A is confounding, for graphics, choose a style that can be clear even if you print in black and white

line 285 what do you mean with "restrictive feeding strategies"?

MEC= I suggest avoiding this kind of abbreviation.

Line 324 ()

I have some doubt about the utility and interpretation of gene expression related to synthesis of milk component in not lactating animals.