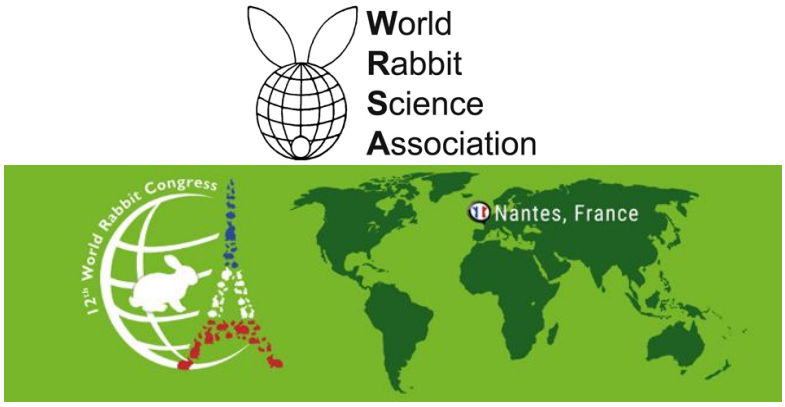

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IMPACT OF DIFFERENT LEVELS OF FEED RESTRICTION DURING FATTENING PERIOD ON GROWTH PERFORMANCES AND MAMMARY GLAND DEVELOPMENT IN MID-TERM PREGNANT DOES

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ABSTRACT

The aim of this study was to evaluate if different feeding practices during the fattening period would have an incidence on doe growth and mammary gland development during first pregnancy. The study tested two levels of feed restriction between 5 and 9 weeks of fattening in does, with one group (G1) receiving a stricter feeding restriction than the other group (G2). Both groups were then managed the same way until first AI and first gestation. Growth and feed conversion were followed every week until 19 weeks of age. At 14 days of gestation, 10 does from each group were euthanized. Mammary tissues were analyzed by histology. Gene expression of milk proteins or lipid enzymes were measured by RT-PCR in the mammary epithelial tissue. Feeding strategies affected body weight curves during growth, but did not affect final BW at AI, nor fertility rate. Histological examinations of mammary tissues revealed no significant difference in tissue morphology between the 2 groups. Milk protein and lipid enzyme FASN genes were significantly increased in the less strict feeding group G2 ($P<0.01$), suggesting increased milk protein and lipid synthesis in the mammary epithelial tissue of does receiving higher feeding allowance in post-weaning period. Altogether, those findings indicate that feeding strategy in early stage of reproductive life in rabbit doe can influence mammary development in first gestation with potential impact on future subsequent lactation.

Key words: feed restriction, mammary gland, mid-pregnancy, milk protein, young rabbit female.

INTRODUCTION

Optimizing rabbit does preparation during its early life to improve reproductive performance is a major challenge for breeders, both on welfare and economic points of view. In modern rabbit production, post-weaning does are often reared the same way as the fattening animals, with serious feed restriction and non-optimal diet to meet specific requirements of those reproductive animals. Feed restriction during rearing period allows uniformity in body weight, limits fattening and decreases mortality around parturition (Rommers et al., 2001). Nevertheless, restricted rabbit females start their first gestation and lactation with a deficient energy level. However, the effect of management and rationed diet around this critical early life period have not been extensively studied in rabbit female (Rebollar et al., 2011; Martinez-Paredes et al., 2012; Pascual et al., 2013).

Mammary gland development is initiated during fetal life and continues over a long period of time (pre-, and post-pubertal periods) and reproductive cycles (pregnancy and lactation). Mammary epithelial growth and differentiation occur under stage-dependent hormonal and environmental control, among which nutrition can play a critical role (Hennighausen and Robinson, 2005; Hue-Beauvais et al., 2011). Thus, breeding factors, and management during post-weaning period, can potentially influence mammary development and future lactation efficiency in female rabbits.

The present study was designed to investigate the impact of two common feed restriction strategies and nutrition management encountered in commercial breeders on growth performances and mammary development during first gestation, with potential impact on future subsequent lactation.

MATERIALS AND METHODS

Animals and feeding strategies

Thirty-eight Hyplus PS19 females were housed in a commercial indoor facility under controlled light conditions and temperature. All rabbits received the same post-weaning commercial diet (2,350 kcal of digestible energy, 15.2 % of crude protein, 17% of celluloses, 0.56% of digestible lysine).

At weaning (35 days of age), the rabbits were randomly divided into two groups according to body weight (832 ± 61 g) and housed in cages of 2. Two different feeding strategies were applied between 5 and 9 weeks of age with one group (G1) receiving a stricter feeding restriction than the other group (G2) (see Table 1 for details). Before puberty, at 9 weeks of age (63 days of age), both groups were fed ad libitum for 3 weeks.
At 12 weeks of age (84 days), rabbits were housed individually. Both groups received 150 g per day of feed, with a feeding flushing (ad libitum) 6 days before artificial insemination (AI; at 130 days of age) to 2 days after AI. At the time of insemination, an injection of GnRH was administered to trigger ovulation.
Feed intake and body weight (BW) were monitored on a weekly basis.

Table 1: Feeding plan

Period of growth	Mean quantity of feed (g) per group and per day	
	G1	G2
35-41d	73	93
42-48d	87	111
49-55d	101	128
56-62d	115	146
63-83d	ad libitum	
84-123d	150	
124-133d (Flushing AI)	ad libitum	
134-144d	150	

Autopsies, mammary gland sampling, histological and quantitative RT-PCR analyses.

At 10 days of gestation, 10 pregnant does per group were chosen on the average body weight at AI and transported to INRA facility, at UMR GABI (Jouy-en-Josas, France). Does were then kept for 4 more days in individual cages, fed with the same diet at 150g/day. On day 14 of pregnancy, all 10 does per group were sacrificed by exsanguination after electronarcosis and the left lower mammary gland from each animal was excised and dissected. Mammary gland tissue was either processed for histology or snap-frozen (epithelial tissue) and stored for later RNA isolation. For histological analyses, samples were processed and analyzed as previously described (Hue-Beauvais et al., 2019). The areas occupied by mammary epithelial tissue, adipose tissue, mammary ducts or connective tissue were measured. For gene analyses, total RNA from mammary epithelial tissue was isolated, and gene expressions of FASN (Fatty Acid Synthase N), SCD (Stearoyl-CoA Desaturase), WAP (Whey Acidic Protein), α-lactalbumin and Casein κ were quantified by real-time Quantitative PCR as previously described by Hue-Beauvais et al. (2019).

Statistical Analysis.
Statistical analysis of data was conducted using SPSS 19.0. On each period, body weight data were analyzed with a repeated measure mixed model (rabbit as random factor, group and week as fixed factors) and ARH1 structure of covariance. ADG and FCR were analyzed by time period with one-way analysis of variance (ANOVA). In all statistical tests, P values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Breeding factors, and management during post-weaning period can influence mammary development and future lactation efficiency in female rabbits. In the present study, the impact of two common feed restriction strategies and nutrition management was investigated on growth performance, and mammary development during first gestation.
Growth parameters and feed consumption are shown in Tables 2 and 3. At the beginning of the trial, mean BW of rabbit does was 832 ± 61 g. Feeding strategies affected body weight curves during growth, but final body weight at AI was not significant between groups. Feeding strategies did not affect BW at first fertile AI, which is in accordance with the BW recommendation to first time inseminated nulliparous does to achieve good results. Fertility rate at AI was also similar between the 2 groups and reached 100%. No mortality was observed in the 2 groups. Average daily gain (ADG) during the first restriction feeding period (i.e. 35-63 days) was higher for G2 ($P<0.05$), in accordance with higher feed allowance. When both groups were fed ad libitum in the second period (63-84 days), ADG was similar between groups. From 84 to 126 days, when both groups were restricted to 150g/day/doe, does from the stricter group (G1) exhibited higher ADG than the does from G2 ($P<0.05$). Those results illustrate that both feeding strategies

allowed the does to reach the same BW at AI, but that timing of body weight gain was not the same between the 2 groups.

Table 2: Body weight during growing period, before insemination

Periods	Age (d)	G1	G2	SEM	P-value
Two levels of feed	42	1087	1161	6.8	<0.001
	49	1321	1413	11.4	<0.001
	56	1666	1741	18.9	<0.001
	63	1818	1954	21.9	<0.001
Ad libitum	70	2112	2178	21.3	<0.001
	77	2347	2462	21.9	<0.001
	84	2589	2730	26.8	<0.001
150g/d	90	2737	2837	30.8	0.010
	98	2911	3018	32.6	0.021
	105	3025	3127	35.8	0.130
	112	3174	3266	36.1	0.102
	119	3318	3392	37.8	0.265
	126	3431	3410	41.5	0.875

Data presented are LS-Means, with standard deviation (SEM) (n=19/group).

Table 3: Zootechnical performance per growth periods

	G1	G2	SEM	P-value
35-63 d: Two levels of feeding restriction				
ADG	33.9	38.6	0.70	<0.001
ADFI	*95*	*121*		
FCR	2.82	3.15	0.06	<0.001
63-84 d: Ad libitum for the 2 groups				
ADG	36.6	37.4	1.20	0.624
ADFI	*156*	*158*		
FCR	4.31	4.31	0.15	1.000
84-126 d: 150g/d for the 2 groups				
ADG	20.1	15.9	0.87	0.002
ADFI	*150*	*150*		
FCR	7.61	10.28	0.64	0.005

Data presented are LS-Means, with standard deviation (SEM) (n=19/group). ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio

In order to investigate if those 2 different feeding strategies, during doe growing period, would impact tissue development in mammary gland during first gestation, mammary gland tissue was examined at 14 days of gestation in 10 pregnant does per group. Prolificacy of rabbit females from both groups did not appear to be affected by feeding strategies. Indeed, at autopsy, no significant difference was observed between groups for number of fetuses, viable fetus nor hemorrhagically fetuses (dead) (data not shown).

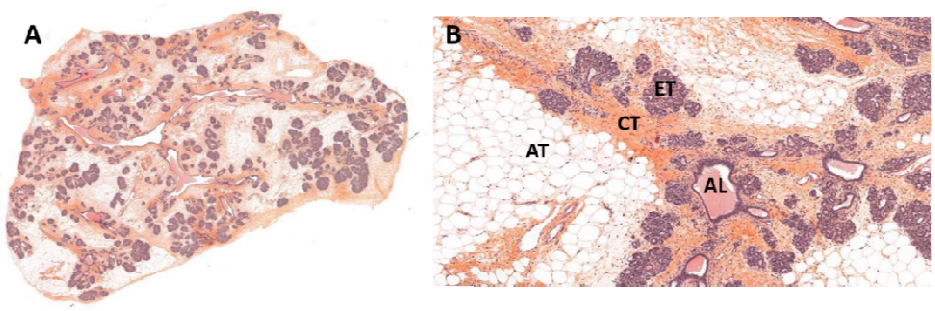

Figure 1: Representative histological cryosection of mammary glands on day 14 of pregnancy and tissue analyzed. Hematoxylin & Eosin staining. Representative scans of total (A) and low magnified (B) sections. ET: epithelial tissue; AT: adipose tissue; CT: connective tissue; AL: alveolar lumina. Figure credit: INRA, UMR GABI (Jouy-en-Josas, France).

Table 4: Relative percentage of the different mammary tissues

	G1	G2	SEM	P-value
Connective tissue %	65.19	57.90	3.07	0.103
Adipose tissue %	12.69	19.78	3.65	0.163
Epithelial tissue %	20.34	21.52	1.71	0.641
Alveolar lumina %	1.40	0.80	0.25	0.098

Histological examinations of thin sections of mammary tissue from both groups were performed on day 14 of pregnancy (Figure 1). Morphological differences were evaluated using a quantitative analysis based on the ratio between adipose tissue, connective tissue, epithelial tissue or alveolar areas per section and total section areas (Table 4). This analysis revealed slight differences between the 2 groups with a tendency for increased alveolar lumina areas and connective tissue in the most fed restricted group G1. Nevertheless, no dramatical disorganization in tissue morphology was observed between the 2 feeding strategies.

Table 5: Expression of milk protein and lipid metabolism enzymes in the epithelial mammary gland tissue

	G1	G2	SEM	P-value
Milk protein synthesis				
k-casein	24131	72376	17585	<0.001
WAP	24910	79660	27733	<0.001
alpha-Lactalbumine	501	1430	314	0.004
Milk lipidic enzymes				
FASN	570	3127	701	0.001
SCD	5726	4132	812	0.110

Expression of gene, quantified at day 14 of gestation, by real-time RT-PCR. Data are means ± SEM (arbitrary units).

To determine whether feeding strategies in pre-puberty might affect the expression of key genes involved in milk protein synthesis or lipid metabolism, real-time RT-PCR experiments were performed in microdissection of epithelial mammary gland tissue (Table 5). The 3 milk proteins tested were significantly increased in the less strict feeding group G2 ($P<0.01$), suggesting increased milk protein synthesis in the mammary epithelial tissue from G2. FASN was also significantly increased in this G2, suggesting increased fatty acid synthesis in the mammary epithelial tissue from G2 rabbits on day 14 of gestation. Those results in gene expression might indicate a better commitment toward lactation production with G2 strategy, i.e. higher feeding allowance in post-weaning period.
Altogether, those findings indicate that feed restriction strategies tested do not negatively impact growth nor mammary gland morphology.

CONCLUSIONS

By subjecting female rabbits to 2 different feeding strategies in prepuberty and by evaluating their mammary development on day 14 of pregnancy, we were able to show that feeding strategy in early stage induced significant changes in morphological and functional tissues, which could perhaps lead to better lactation production for the less strict restriction. Those findings indicate the need for more investigations on nulliparous management and nutrition post-weaning in order to provide specific recommendations to potentiate fertility and lactation quality and productivity of young female rabbits.

ACKNOWLEDGEMENTS

The authors acknowledge the staff of Hypharm facility for its care of the animals. They also thanks INRA GABI team and especially Dr Hue-Beauvais, Dr Charlier and Dr le Provost for the mammary gland investigations. Philippe Boniface from CCPA group is also acknowledged for the help in statistical analyses of growing performances.

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Impact of different levels of feed restriction during fattening period on growth performances and mammary gland development in mid-term pregnant does

D. Gardan-Salmon, **M. Maupin**, K. Bébin, R. Robert

World Rabbit Congress 2021, Nantes

Context and Objectives of the study

- Optimization of rabbit does preparation for their reproductive performance
- Feed restriction during rearing period allows uniformity in body weight, limits fattening and mortality around parturition (Rommers et al., 2001)
- Serious feed restriction in post-weaning are often applied:

  → deficient energy level/status at first mating?

  → potential impact on their milk production?

Objective : Investigate the impact of two common feed restriction strategies:

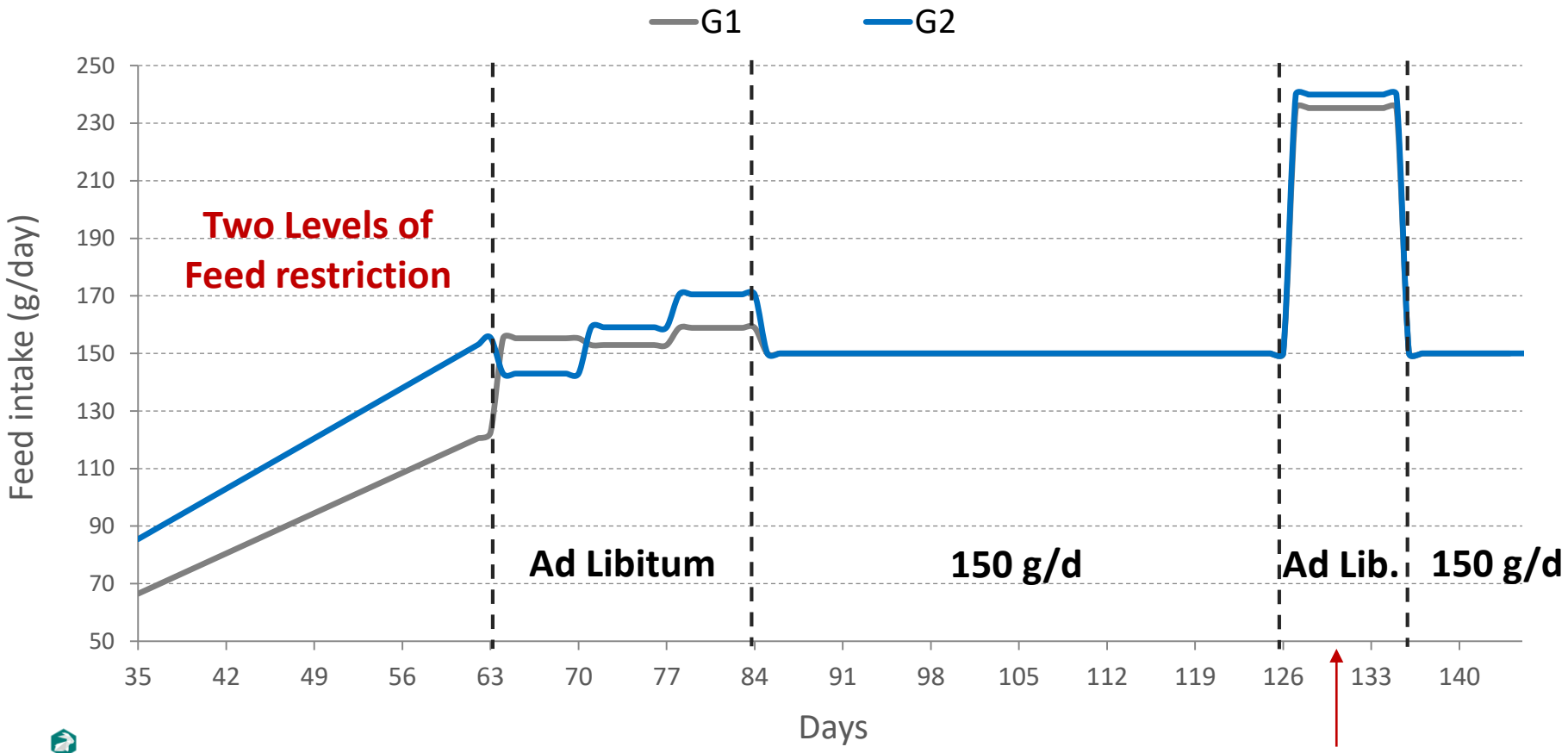
- **on growth performances**
- **on mammary development during first gestation**

Materials and Methods – Feed restriction plan

38 weaned Hyplus females

Weaned at 35 days
832 ± 61 g

2 groups of feed restriction in fattening period (35-63d):



Note: Artificial insemination (AI) at 130 days of age

Materials and Methods – Measurements

- Feed intake and body weight (BW) were monitored on a weekly basis
- Day 14 of gestation: 10 pregnant does sacrificed / group
- Mammary gland tissue studied by INRAE

Mammary gland tissue

Histological analyses

Areas measurement of epithelial tissue, adipose tissue, mammary ducts or connetive tissue

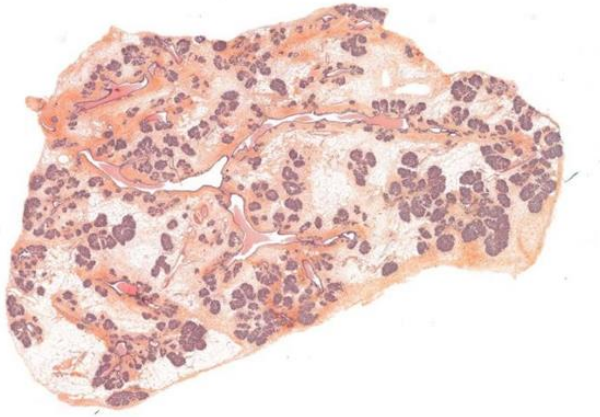


Figure credit: INRA, UMR GABI (Jouy-en-Josas, France)

Real-time Quantitative PCR

on epithelial tissue for WAP, α-lactalbumine, Casein κ and FASN

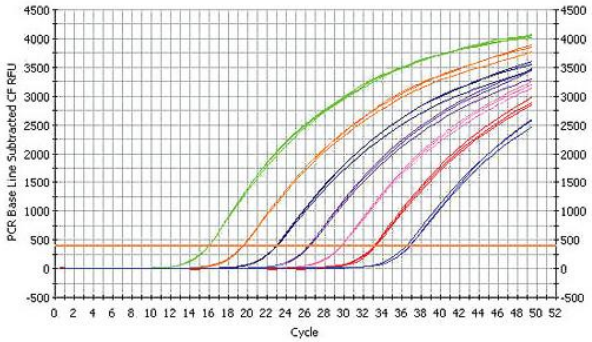
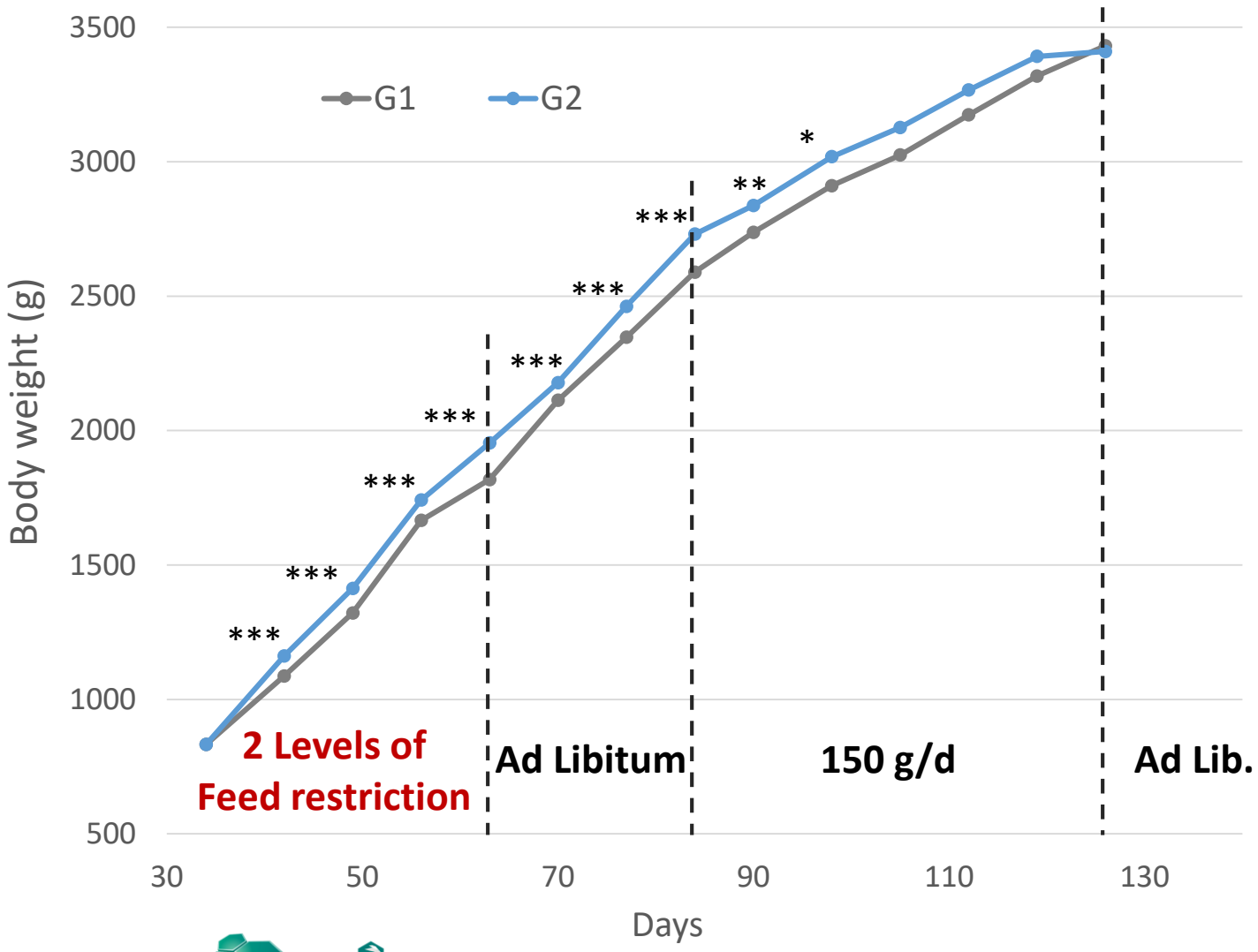


Figure credit: Bio-Rad Laboratories, Inc.

Results - Growth performances



- Two levels of feed restriction:
  - Differences in body weight curves during growth ($P<0.05$)
  - Even during the common feed strategy (63-130d)
- But final body weight was not significant between groups

Results - Mammary gland investigations

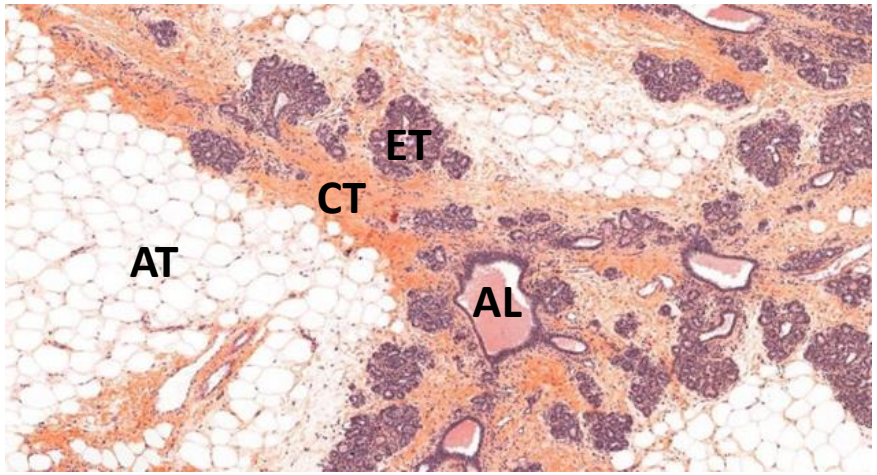
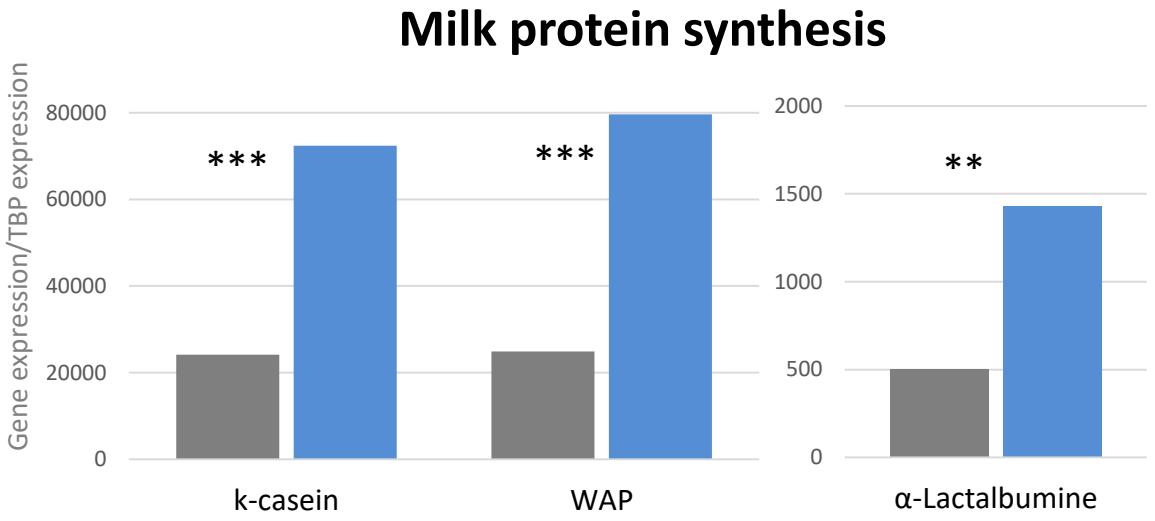
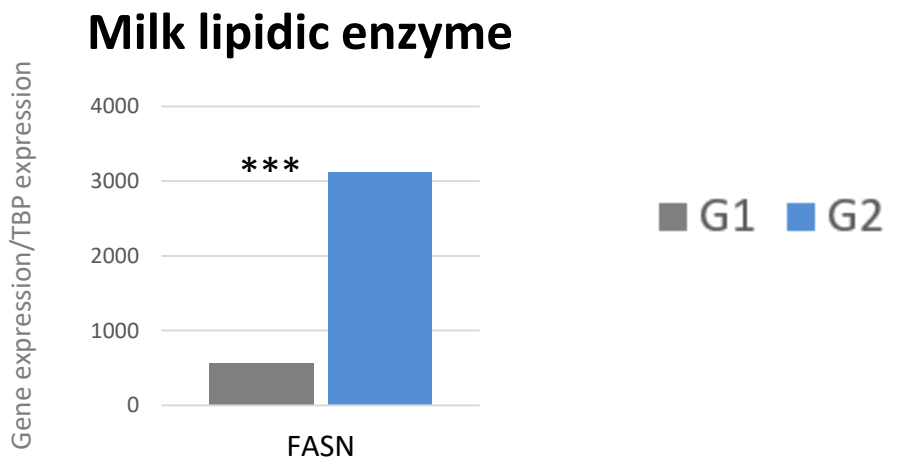


Figure credit: INRA, UMR GABI (Jouy-en-Josas, France).

	G1	G2	P-value
Connective tissue (CT), %	65.2	57.9	0.103
Adipose tissue (AT), %	12.7	19.8	0.163
Epithelial tissue (ET), %	20.3	21.5	0.641
Alveolar lumina (AL), %	1.4	0.8	0.098

- Slight differences between the 2 groups
- No major impact in tissue morphology





- Increased gene expressions in group G2 (P<0.01)

Conclusions

- Feed restriction strategies in fattening period influence the growth curve until AI
- No negative impact on mammary gland morphology at d14 gestation
- Higher feeding allowance in post-weaning period increases expression of genes involved in milk lipid and protein production
- More investigations are needed on nutrition post-weaning to provide specific recommendations for the nulliparous management

THANK YOU FOR YOUR ATTENTION

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