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MILK DIGESTION IN THE YOUNG RABBIT: METHODOLOGY AND FIRST RESULTS

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Abstract: This study aims to determine the digestibility of milk by the young rabbit (21-25 d old), taking into account the increment of digesta content and urine excretion. Nineteen litters of 9 young rabbits 21 to 25 d old were used: 12 litters (S group) fed exclusively with milk using controlled suckling, and 7 litters (Control group) with free suckling and access to the pelleted feed of the doe. The faecal digestibility of milk dry matter (DM) was measured between 21 and 25 d of age, for S litters housed from 15 d of age in a metabolism cage separately from their mother. Between 21 and 25 d, the milk intake, faeces and urine excretion were controlled daily, and the mean increment in digesta content was measured by comparing digesta weight of the whole tract at 21 and 25 d of age (one kit per litter). The increment in digesta content from 21 to 25 d averaged 77% (+8.5 g), sourcing mainly from stomach and caecum contents increase (+57 and +120% respectively). The mean increase for the dry content of the gut (from 21 to 25 d) was 1.75 g DM/kit, and was considered as non-digested to calculate the digestibility coefficient of the milk. The milk intake averaged 30 g/d/kit (7.9 g DM/d kit). No faecal excretion was recorded between 21 and 25 d. From the milk intake and increment in digesta content, the corrected digestibility of the milk dry matter reached 94% (minimum=92.9%, maximum=95.6%). The daily urine excretion averaged 5.1 mL/kit, corresponding to 1.2 g DM/kit. Therefore, the corrected DM retention coefficient of the milk was 79.5%. The quantity of nitrogen excreted in urine was low (0.06 g/d kits), thus the corrected nitrogen retention coefficient for milk reached 82% and the nitrogen retained (corrected) reached 0.44 g/d kit. Accordingly, the amount in metabolisable protein for the milk was 90 g/kg (fresh). The corrected energy retention coefficient was estimated to 95.8%, for a crude energy concentration estimated at 28.14 MJ/kg DM for the milk. Thus, the energy retained (corrected) reached 223 kJ/d kit and the content in metabolisable energy for the milk was 26.94 MJ/kg DM.

Key Words: young rabbit, digestion, milk, methodology.

INTRODUCTION

As in other mammals, the weaning period is crucial for the young rabbit, as the gastrointestinal functions develop sharply from 3rd week of age when the rabbit starts to eat solid feed. However, the digestive capacity of the young rabbit, essential to determine the nutritional requirements and to further prevent digestive diseases, has been extensively studied after weaning (Carabaño *et al.,* 2010), and only a few studies look at the digestion before weaning, when young rabbits consume milk and solid pelleted feed (Gallois *et al.,* 2005, 2008). For instance, the whole digestive tract efficiency increased by 10% before weaning (32 d old) and remained steady (organic matter) or decreased (lipids, protein) after weaning (Gidenne *et al.,* 2007). Only the study by Parigi-Bini *et al.* (1991) assessed the digestion for suckling rabbit, and, by comparative slaughter technique and multiple regression method, they estimated that the milk dry matter was totally digested. However, in this study, the suckling rabbits also consumed solid feed (from doe feeder), and no results were obtained for the digestion of exclusively milk-fed rabbits. Furthermore, around weaning the digestive tract is developing sharply, and Gallois *et al.* (2005) found a 72% increase in digesta content between

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21 and 28 d of age (+38.2 g) for suckling rabbits also consuming solid feed. This increment in digesta content must be taken into account in the digestibility calculation.

Our study thus aimed to determine the digestion of the milk only, for 3 wk-old rabbits exclusively milk-fed and housed in metabolism cages separately from the doe. We also aimed to adjust a procedure to correct the calculation of digestibility coefficient by measuring the increment in digesta content during the digestibility period (21-25 d of age).

MATERIALS AND METHODS

Experimental design, animals and housing

The study was conducted in accordance with the French legislation on animal experimentation and ethics, and the senior researchers were authorised by the French Ministry of Agriculture to conduct experiments on living animals at the INRA facilities of PECTOUL, Auzeville, France.

This study used 19 litters of 9 young each, obtained with a 3-way cross (female INRA1067×Grimaud line PS19, male Zika maternal quality) and from multiparous does (parity between 2 and 4). Does and litters were housed in metabolism cages (H: 29 cm, L: 47 cm), in an indoor breeding unit (light schedule: 07:00-19:00; 21°C). Does were freely fed a commercial pelleted feed and artificial insemination was performed 11 d after delivering.

At 17 d of age, the litters were divided into 2 groups. Seven litters were assigned to the control group (C) and remained classically housed in the doe cage with their mother and with free nursing and access to the doe feed. Twelve litters were assigned to the “suckling only” group (S), and housed in a metabolism cage (same model as the control group but without nest box) without access to solid feed, separately from their mother (housed adjacent). Daily suckling was achieved at 9:00 am by introducing the mother into the litter cage, from 17 d to 25 d of age. The cages were equipped with a 1 mm mesh to collect the faeces, and with a stainless funnel adapted under the cage to collect urine from 21 to 25 d of age. The growth performances were measured at 15, 21 and 25 d in the 2 groups. Health status was checked through a daily clinical examination of the animals. This consisted of monitoring the animals for clinical signs of digestive disorders such as diarrhoea, caecal impaction, suspicion of ERE (Epizootic Rabbit Enteropathy) or other pathologies (respiratory problems, injuries …).

Procedure for urine collection and milk digestibility measurement

A period of adaptation to controlled nursing was managed between 17 and 21 d of age. This also should allow the removal of digesta sourced from a potential solid feed intake before 17 d of age.

The digestibility period covered 4 consecutive days: from 21 to 25 d of age. Each morning, milk intake was first measured by weighing the doe before and after milking. Then, potential hard faeces excretion was checked and the urine was quantitatively collected in a beaker containing 40 mL of sulphuric acid (10% v/v) to prevent ammonia volatilisation (Udert *et al.,* 2003), as follows: before urine collection, the litter was removed from the cage and the funnel was rinsed with 50 mL of sulphuric acid solution (10% v/v). The weight and volume of urine collected were measured daily, and 10% of the urine daily collected was stored at –18°C for subsequent N analysis. Milk was sampled from 3 does of the control group at the 18th day of lactation, then stored at –18°C, for further analysis in dry matter, crude fat and nitrogen.

Procedure to correct the digestibility coefficient and calculations

The digestive content is increasing sharply when the dry feed intake starts (around 17 d of age), for instance the stomach content tripled from 17 to 35 d (Orengo and Gidenne, 2007). This increment in digesta weight must be taken into account when calculating the digestibility coefficient to respect the balance between intake and excretion. Therefore, one kit of each litter of the S group was sacrificed (at 09:00 before milking) at 21 d of age and at 25 d (after the last urine collection) to determine the weight of the digesta content (stomach, small intestine, caecum and colon). Then, for each rabbit, a pooled sample (from each segment) was dried (24 h, 103°C) to calculate the dry matter (DM) content of the whole gut (DCG) at 21 and 25 d of age. The mean variation in DCG between 25 and 21 d was considered as excreta to calculate the corrected digestibility coefficient of the milk. As we did not detect any faecal excretion between 21 and 25 d, the formula to calculate the milk DM digestibility is: (DMim-DCG)/DMim, with DMim corresponding to the DM intake from milk only.

Nitrogen concentration of urine sample (one per litter) was analysed to calculate the nitrogen excretion over the 4-d collection periods (21-25 d of age). The equation of Parigi-Bini and Cesselli (1976) was used to calculate the urinary energy excretion using nitrogen excretion: $Y=-0.72+12.37X$, where Y is the urinary energy excretion (kcal/d) and X is the urinary nitrogen excretion (g/d). The energy concentration in milk was estimated during the 4th week of lactation to 28.14 MJ/kg DM (7.32 MJ/kg), according to Maertens *et al.* (2006), while the energy concentration of digesta was estimated to 15.06 MJ/kg DM, similar to that in faeces of 4-wk-old rabbit (Gallois *et al.,* 2008). Therefore, coefficients of retention for nitrogen and energy were calculated as: intake–(urine excretion+digesta content increment).

Chemical analysis

The following chemical analyses were performed on milk, digesta content and urine according to ISO methods and considering the recommendations of the European Group on Rabbit Nutrition (EGRAN, 2001): dry matter (DM) (ISO 6496, 1999), nitrogen (N) (Dumas method, ISO 16634, 2004) using a Leco auto-analyser (model FP-428, Leco Corp., St Joseph, MI, USA). Crude fat was determined in milk samples using Soxtec system H+ (after acid hydrolysis pre-treatment) according to the method described by Alstin and Nilsson (1990).

Data Analysis

Two litters from the S group were removed from data analysis due to milking problems with the doe. Another litter showed a digesta content weight 2 times higher than the other 9 values of the S group, at 21 and 25 d of age, and this was the only litter with a small faecal excretion during 2 d after trial start (2.4 g DM at 21 d, and 0.6 g DM at 22 d old). We assume that this litter already consumed pelleted feed with the doe at 17 d of age. We thus removed it from digesta content analysis and from the milk digestion calculation.

Live weight data for litter and doe and data of digestive segment weight of kits were compared for age effect, according to a mono-factorial variance analysis (procedure GLM under SAS). Milk intake and milk digestion data were original and obtained only for the S group, thus we give the mean value associated with the variation coefficient and the minimum and maximum values observed.

RESULTS AND DISCUSSION

Growth of litters and doe live weight variation during milking period

In the control group, the live weight of the litters ranged within the classical values observed for this rabbit line (Table 1). They were similar to that of S group at the start of the trial (mean=240 g/kit at 15 d of age), but were 10% heavier at 21 d and 18% heavier at 25 d ($P<0.01$). The growth rate was 33% higher for the control group during the

Table 1: Live weight and growth of the young rabbits, from 15 to 25 d of age, according to the suckling procedure.

Group	S	C	rVC (%)	*P*-value
Live weight (g/kit)				
15 d	238	243	9.1	0.616
21 d	296	325	9.1	0.057
25 d	370	436	8.9	<0.01
Weight gain (g/d)				
15-21 d	9.8	13.6	27.4	0.024
21-25 d	18.4	27.7	28.7	<0.01
15-25 d	13.2	19.3	14.6	<0.001

S, suckling only, separate housing for the litter and the doe, without access to solid feed (10 litters); C, control, free suckling and access to solid feeds (7 litters).

rVC: Residual variation coefficient, calculated as (root mean square error)/mean, and expressed in %.

Table 2: Doe live weight variation between 15 and 25 d of lactation.

Group	S	C	rVC (%)	*P*-value
Live weight (g)				
15 d	4534	4701	9.3	0.401
21 d	4163	4632	9.0	0.017
25 d	4284	4563	10.1	0.17
Weight gain (g/d)				
15-21 d	–61.8	–5.4	60	<0.001
21-25 d	30.2	–20.0	312	<0.01
15-25 d	–25.0	–11.2	77	0.079

S, suckiling only, separate housing for the litter and the doe, without access to solid feed (10 litters); C, control, free suckling and access to solid feeds (7 litters).
rVC: Residual variation coefficient, calculated as (root mean square error)/mean, and expressed in %.

21-25 d period ($P<0.01$). This result was expected, as control group kits were able to access the solid feed in their mother's feeder. According to the literature, the solid intake ranged between 1 and 5 g/d (Gidenne *et al.,* 2015) for freely nursed litters. In parallel, the weight of the doe decreased by 20 g/d during the 21-25 d period for the control group (Table 2), while it increased by 30 g/d in S group. This may suggest that the nursing behaviour of the S does was impaired by our procedure to control the milk intake of the litter (daily transfer from their cage to the litter cage and back). Similar effects of controlled milking on litter growth have already been described (Ubilla *et al.,* 2000; Zhang *et al.,* 2018).

Development of the digestive tract during the digestibility period

At 21 d of age, the whole fresh content of the gut corresponded to 3.7% of the live weight, and 5.3% at 25 d of age (Table 3). Accordingly, between 21 and 25 d the whole digesta content rose by 77% (+8.5 g), although the rabbits were fed only with milk. This increase was sourced mainly from stomach and caecum contents increase: +57% and +120%, respectively. Within 4 d the relative weight of the stomach content decreased from 55 to 49% ($P<0.001$), while for the caecum it increased from 33% to 40% ($P<0.001$). Similarly, Gallois *et al.* (2005) described a similar increase for the stomach content fresh weight (+53%) between 21 to 28 d of age, but a sharper increase for the caecum (+350%), as the young rabbit consumed a significant amount of solid feed after 21 d of age. However, a large inter-individual variability (25 to 50%) was observed for all criteria, although the live weight variability of those sacrificed kits was low (5.7%).

The dry matter concentration of the whole digestive content (pooled sample of the different segment) averaged 20.6%, with no significant difference between 21 and 25 d ($P=0.151$). The total dry content of the gut averaged 2.3 g per kit at 21 d of age and reached 4.0 g at 25 d. Thus, the mean increase for the dry content of the gut (DCGi)

Table 3: Changes in digestive content weight of the exclusively milk-fed young rabbit[1] gut (group S), during the digestibility measurement period.

	21 d old	25 d old	rVC (%)	*P*-value
Live weight (g/kit)	291	371	5.7	<0.01
Weight of content (g fresh matter)				
Stomach	6.1	9.6	23.6	<0.01
Small intestine	0.7	1.4	43.5	>0.01
Cæcum	3.6	7.9	38.7	<0.001
Colon	0.6	0.6	53.3	0.970
Total fresh content	11.0	19.5	23.0	<0.001
Total dry content (g)	2.3	4.0	31.7	<0.001

[1]Mean value for 9 kits sacrificed at 21 d, and 9 kits at 25 d (after digestibility measurements), only suckling and without access to solid feed (group S).
rVC: Residual variation coefficient, calculated as (root mean square error)/mean, and expressed in %.

Table 4: Milk consumption of exclusively milk-fed litters (group S), from 21 to 25 d of age, and digestibility of the dry matter from the milk[1].

	Mean*	VC (%)	Min	Max
Period 21 to 25 d old				
Milk intake (g/d kit)	29.6	4.2	23.8	38.0
Milk intake (g dry matter/d kit)	7.9	12.8	6.2	9.9
Dry matter digestibility, corrected for DCGi[2] (%)	94.4	0.8	92.9	95.6

[1]No faecal excretion was detected for the 9 litters between 21 and 25 d.

[2]Mean increase for the dry content of the gut (DCGi) from 21 to 25 d old (g DM/kit)=1.75 g (see Table 3).

*Mean calculated on 9 litters of 8 kits. VC: Variation coefficient (standard deviation/mean) expressed in %.

from 21 to 25 d of age (g DM/kit) was 1.75 g, and was considered as non-digested to calculate the digestibility coefficient.

During digesta sampling, we found some hard faecal material in the distal colon for 7 out of 9 kits at 21 or 25 d of age. These hard faeces were not retrieved on the collection sieve, but some faecal material was found in the stomach in 2 kits at 21 d. This suggested that the young rabbit would still practice coprophagy at 3 wk of age, under our controlled milking procedure with a separate housing for doe and litter.

Milk intake and digestion

The chemical composition of the milk was as classically reported (Lebas, 1971; Maertens *et al.,* 2006), with a dry matter level of 260 g/kg, ash content of 85 g/kg, nitrogen concentration of 66.2 g N/kg DM corresponding to 422 g/kg DM of crude protein (using a conversion coefficient of 6.38), and a crude fat content of 424 g/kg DM. Therefore, the sum of protein and lipid corresponded to almost 85% of the milk composition. The crude energy concentration was calculated from the chemical composition and literature data (Maertens *et al.,* 2006) and estimated at 28.85 Kj/kg DM, corresponding to 7.50 Kj/kg.

The milk intake averaged 30 g/d and per kit (7.9 g DM/d kit, Table 4), which corresponded to a total milk production of 240 g/d (8 kits per litter). This ranged within the value reported in the literature for European commercial doe lines (Maertens *et al.,* 2006; Savietto *et al.,* 2014).

From the dry matter milk intake and increment in digesta content, we calculated that the corrected digestibility of the milk reached 94.4%, with a minimum value of 92.9% and a maximum at 95.6%, thus corresponding to a relatively low variability (0.8%). In contrast, for young rabbits (between 21 and 26 d old) fed milk and solid feed, Parigi-Bini *et al.* (1991) estimated (multiple regression) that milk was totally digested (100.2%), while the solid feed was digested to 64.4%.

The urine excretion averaged 5.1 mL/d kit and had 50% variability among litters, although the DM urine excretion had a 11% variability and averaged 1.2 g DM/d/kit (Table 5). Besides, no significant relationship was found between the urine volume (21-25 d) and the milk intake (R^2=0.12, n=9, P=0.75), while weight gain was logically correlated

Table 5: Urine excretion of the exclusively milk-fed litters (group S), from 21 to 25 d of age, and dry matter retention of the milk[1].

	Mean*	VC (%)	Min	Max
Period 21 to 25 d old				
Urine excretion (mL/d kit)	5.1	48	1.7	10.2
Urine excretion (g dry matter/d kit)	1.2	11	1.0	1.4
Dry matter retention (corrected for DCGi[2]) (%)	79.5	2.8	77.1	83.1

[1]No faecal excretion was detected for the 9 litters between 21 and 25 d.

[2]Mean increase for the dry content of the gut (DCGi) from 21 to 25 d old (g dry matter/kit)= 1.75g (see Table 3).

*Mean calculated on 8 litters of 8 kits (for one litter, the urine excretion was unavailable). VC: Variation coefficient (standard deviation/mean) expressed in %.

Table 6: Digestibility and retention of nitrogen from the milk for the 3-wk-old rabbit exclusively milk-fed (group S).

	Mean*	VC (%)	Min	Max
Nitrogen balance, period 21 to 25 d				
Nitrogen intake from the milk*[1] (g/d kit)	0.53	10.4	0.49	0.65
Nitrogen excretion from urine*[1] (g/d kit)	0.06	33.5	0.04	0.10
Nitrogen digestion and retention				
Corrected digestibility of nitrogen** (%)	94.1	0.6	93.5	95.2
Nitrogen retention coefficient (%)	87.8	5.5	79.2	93.0
Corrected nitrogen retention coefficient (%)**	81.9	5.5	72.7	88.1
Nitrogen retained (g/d kit) (corrected)**	0.44	15.9	0.35	0.58
N retained: N digestible coefficient** (%)	87.0	5.9	77.8	92.6
Metabolisable protein[2] of the milk (g/kg)	90	7	80	97
Metabolisable protein[2] of the milk (g/kg dry)	346	7	307	372

*Mean calculated on 8 litters of 8 kits (for one litter, the urine excretion was unavailable).

**Correction for the increase in the gut nitrogen content, from 21 to 25 d of age= 0.13 g N/kit.

[1]No faecal excretion was detected for the 8 litters between 21 and 25 d.

[2]Nitrogen was converted in protein using a coefficient of 6.38.

VC: Variation coefficient (standard deviation/mean) expressed in %.

with milk intake (R^2=0.70, n=9, P<0.010). When accounting for the urine excretion, the corrected DM retention coefficient of the milk was 79.5%, with a 2.8% variation.

As the quantity of nitrogen excreted in urine was low (0.06 g/d/kits), the corrected nitrogen digestibility of the milk reached 94% (Table 6) and varied very little (0.6%). The corrected nitrogen retention coefficient for milk was much lower (82%) and more variable, and the nitrogen retained (corrected) reached 0.44 g/d kit. In comparison, Parigi-Bini *et al.* (1991) calculated higher coefficients, with a nitrogen digestion of 98.6%, and a nitrogen retention of 94%. Accordingly, we calculated that the content in metabolisable protein for the milk was 90 g/kg (fresh), with a variation coefficient of 7%.

We only estimated the energy digestion and retention, as the mass of digesta and milk samples were too low to perform precise calorimetric measurements. Thus, energy content of milk was assessed from the literature (Maertens *et al.,* 2006), while we assumed that energy content was similar to that of faeces (Gallois *et al.,* 2008). Accordingly,

Table 7: Estimation of the digestion and retention of energy from the milk, for the 3-wk-old rabbit exclusively milk-fed (group S).

	Mean*	VC (%)	Min	Max
Energy balance, period 21 to 25 d				
Energy intake from the milk*[1] (kJ/d kit)	233	10	211	284
Variation in gut energy content, between 25 and 21 d of age (kJ/kit)	26	52.0	1.8	10.8
Energy excretion from urine*[1] (kJ/d kit)	3.2	34	1.9	5.1
Energy digestion and retention				
Corrected digestibility of energy** (%)	97.2	1.5	94.8	98.5
Energy retention coefficient (%)	98.6	0.6	97.6	99.3
Corrected energy retention coeff.** (%)	95.8	0.8	94.6	97.0
Energy retained (kJ/d kit) (corrected)**	223	11.4	199	276
Energy retained: digestible energy coeff.** (%)	98.5	0.6	97.6	99.2
Metabolisable energy of the milk (MJ/kg)	7.00	0.8	6.91	7.09
Metabolisable energy of the milk (MJ/kg dry matter)	26.94	0.8	26.58	27.26

*Mean calculated on 8 litters of 8 kits (for one litter, the urine excretion was unavailable).

**Correction for the variation in the content of energy in the gut, between 21 and 25 d of age =26.1 kJ/kit.

[1]No faecal excretion was detected for the 8 litters between 21 and 25 d.

VC: Variation coefficient (standard deviation/mean) expressed in %.

the corrected energy digestibility of the milk was very high 97% (Table 7), as well as the corrected energy retention coefficient of the milk (95.8%); hence, the energy retained (corrected) reached 223 kJ/d kit. In comparison, Parigi-Bini *et al.* (1991) estimated a slightly higher coefficient for milk energy digestion (99.7%), but a much lower energy retention (86%). It should be noted that the amount of energy excreted in urine (3.2 kJ/d kit) was 50% lower than energy "retained" from digesta increment (6.5 kJ/d kit). Therefore, we suspected that the energy concentration in urine may be underestimated, as it was calculated according to the equation of Parigi-Bini and Cesselli (1976) adapted to growing rabbit at 2 kg live weight. In perspective, energy concentration of urine and digesta (and milk) should be measured in several samples to improve the precision of the energy balance in milk-fed young rabbit. However, we estimated that the content in metabolisable energy for the milk was 7.00 MJ/kg (fresh), with a variation coefficient of 0.8%.

CONCLUSION

Our study presented original results about milk digestion by the young rabbit. Our methodology took into account the increment of digesta content in the 3-wk-old rabbit. Initially recognised as fully digested, we found that after correction for digesta increment, the milk DM digestion however reached 94% and DM retention was 90%. The milk nitrogen retention was relatively high (82%), corresponding to a metabolisable protein concentration of 90 g/kg.

In perspective, our first results on milk digestion could be confirmed by increasing the sample size (involving a higher number of sacrificed kits) to allow further chemical analysis and address the digestion of lipid.

The milk digestion here was measured in 3-wk-old rabbits fed exclusively with milk, and this will allow us to calculate digestibility coefficient for feed before weaning, taking into account this value. However, we cannot exclude digestive interactions between solid feed and milk for rabbits beginning to consume pellets and starting caecotrophy (between 3 and 5 wk of age).

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