



Effect of secondary metabolites of *Euphorbia heterophylla* (L.) klotz. & garcke, and *Moringa oleifera* (Lam). on the milk production of the local female rabbit (*Oryctolagus cuniculus* L.)

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Abstract

The present study aims at comparing the effect of minerals and secondary metabolites of *Euphorbia heterophylla* and *Moringa oleifera* associated with *Panicum maximum* G23 on the milk production of local female rabbit. In total, 18 local female rabbits (*Oryctolagus cuniculus*) of 10 months, all at their second litter and average weight 2.9 ± 0.2 kg, were used. They were mated and randomly divided into three homogeneous lots in individual maternity cages. Each lot was subjected either to a control diet (*Pangran*) consisting of *Panicum maxima* and rabbit pellets or to an experimental diet (*Paneuph* or *Panmor*) consisting of *Panicum maxima* and rabbit pellets in combination with *Euphorbia heterophylla* or *Moringa oleifera*. The milk production of female rabbits and the average daily gain (ADG) related to milk consumption of bunnies at peak lactation were 140.0 ± 31.4 g, 109.6 ± 22.0 g and 131.5 ± 29.6 g and 6.6 ± 1.4 , 6.3 ± 1.2 and 5.8 ± 1.1 g.d⁻¹ for *Paneuph*, *Pangran* and *Panmor*, respectively. The contribution of *Euphorbia heterophylla* and *Moringa oleifera*, induced significantly higher milk production than that of *Pangran* (109.6 ± 22.0 g) ($P < 0.05$). However, the ADGs of bunnies under the three diets were not significantly different ($P > 0.05$). Considering the quantities of milk produced by the three lots of rabbits, *Euphorbia heterophylla* would be a galactogenic plant in the same way as *Moringa oleifera*.

Keywords: secondary metabolite, *Euphorbia heterophylla*, *Moringa oleifera*, *Oryctolagus cuniculus*

1. Introduction

For a long time, Man has been nourished and cured with plants that he had at his disposal against various diseases. It is neither chance nor superstition that has guided traditional medicine to use one plant rather than another, but rather the endogenous knowledge of the people [1]. Indeed, the plant is the site of intense metabolic activity leading to the synthesis of a wide range of active ingredients [2]. The latter give plants their analgesic, soothing, laxative, galactogenic, etc. virtues [3].

In recent decades, a growing interest in plants with a galactogenic or galactogogue effect has been observed in different regions of the world [4, 5]. Indeed, these plants are used to initiate, maintain or increase milk production in mammals or lactating women [6]. In France, fenugreek seeds, leaves and flowers of officinal *Galéga* and seeds, leaves, buds and capsules of milk thistle (*Silybum marianum*) are commonly used to increase milk production in lactating women and animals [7]. In India, the leaves of *Moringa oleifera* are used for their laxative and galactogenic properties [8]. The dried root powder of *Calotropis procera* mixed with water is used to promote

lactation in breastfeeding mothers and is administered to women to facilitate childbirth in Africa [9].

It is in this context that in Côte d'Ivoire, Adepo *et al* (2010; 2017) [10, 11] conducted studies on the galactogenic properties of *Euphorbia hirta* and *Secamone afzeli*. These studies showed the galactogenic power of these plants in rats.

In addition to *E. hirta*, which has been studied by d'Adepo *et al.* (2010; 2017) [10, 11], several other species of the Euphorbiaceae family such as *E. balsamifera*, *E. polycnemoides*, *E. convolvuloides* also possess galactogenic properties [12]. However, there are others such as *E. heterophylla*, whose roots are used in East Africa to treat gonorrhoea or increase lactation in lactating women [13], but which have not yet been sufficiently studied scientifically.

Furthermore, *Euphorbia heterophylla* is a weed with a high crude protein content (more than 16%), and a high content of the polyunsaturated fatty acids C18:3 n-3 (55% of total fatty acids) and C18:2 n-6, (22% of total fatty acids) [14]. Its belonging to the family of Euphorbiaceae, of which several species are galactogenic, and its latex content, characteristic of galactogenic plants [12], suggests that this species also has galactogenic properties. Therefore, it is important to study

its galactogenic properties in comparison with other plants such as *Moringa oleifera*, whose effects on the induction of milk production are well known, in order to diversify the range of galactogenic plants that can be used to compensate for the deficit in milk secretion in lactating women. It is in this perspective that the present study, whose objective is to evaluate the effect of secondary metabolites of *Euphorbia heterophylla* compared to that of *Moringa oleifera* on the quantity of milk produced at the peak of lactation by local female rabbits, is conducted. More specifically, the nutritional value of different diets will be evaluated through the weight growth of the bunnies on the one hand and the effect of secondary metabolites of these fodders on the quantities of milk produced at peak lactation by the female rabbits on the other hand.

2. Material and Methods

2.1 Study area

The study was conducted from April to June 2018, at the experimental farm of the Institut National Polytechnique Félix Houphouët-Boigny (INP-HB) in Yamoussoukro (Côte d'Ivoire), located precisely at 6.5° North longitude and 5.2° West latitude. During the test period, the average values of temperature and relative humidity were $26.6 \pm 1.2^\circ\text{C}$ and $77.85 \pm 3.25\%$ respectively. The average rainfall was 148.0 ± 20.4 mm.

2.2 Biological Material

2.2.1 Animal Material

The study involved 18 local non-pregnant rabbits of the same litter rank. They had an average weight of 3.0 ± 0.2 kg and were 10 months old (Fig 1).

2.2.2 Plant Material

The fodders consisted of *Panicum maximum* G23, *Euphorbia heterophylla* and *Moringa oleifera*, harvested from the northern site of INP-HB (Fig 2).

2.2.3 Technical Material

2.2.3.1 Livestock building

The test was carried out in the rabbit breeding building of the experimental farm of the zootechnics laboratory of the Department of Training and Research-Agriculture and Animal Resources (DFR-ARA) of INP-HB in Yamoussoukro.

It is a 54 m² (6 x 9 m) building, exposed to natural ventilation, photoperiod and temperature. Lighting is natural with artificial complementing provided by two 150 cm neon tubes with a power of 18W which are lit for 14 to 16 hours/24 hours.

2.2.3.2 Livestock Material

-two (2) machetes for mowing fodder and a wheelbarrow for transporting fodder and refuse.

-a broom, two brushes and a bucket were used to clean the livestock building.

2.2.3.3 Weighing Material

-a Hair© brand electronic scale with a maximum capacity of 5000 g (accuracy 1 g) for weighing animals and fodder, followed by a 10 L bucket and two 25 L basins each to hold the animals and feed weighed.

2.2.3.4 Determination of the Nutrient Value of Foods Used

-Bromatological analysis material

The bromatological analysis material consisted of apparatus and glassware for the analysis of dry matter, fat, ash and nitrogen. It consisted of a desiccator (GLASWERK, 250 SAPAIC, WERTHEM) for cooling the various samples in a dry environment. A ball mill (RETSCH S1, Haan, Germany) was used to grind the samples when necessary. A Sartorius A220 S electronic laboratory balance with a maximum capacity of 2100 g was used to weigh the samples. A drying oven from MEMMERT 854, UL80, SCHWABACH, Germany, was used to dry the different samples. A muffle furnace (Nabertherm GmbH, L5/11, Lilienthal, Germany) was used for calcining the samples. A distiller (Buchi 321 Kjeldahl, Flawil, Switzerland) was used for the determination of the total nitrogen (Nx6.25).



Fig 1: Local female rabbit (*Oryctolagus cuniculus*)



Fig 2 a: *Panicum maximum* G23



Fig 2 b: *Euphorbia heterophylla*



Fig 2 c: *Moringa oleifera*

3- Methods

3.1 Experimental device

The female rabbits were randomly and individually placed in maternity cages of dimensions (60 x 40 x 28 cm), each with a nest box 40 cm long, 25 cm wide and 30 cm high (Fig 3).

Underneath all these cages were mosquito netting to collect fallen food. These nets were held 20 cm above the ground with small metal supports to facilitate the flow of urine. Each of the cages was equipped with a homemade metal feeder and drinking trough.

3.1.1 Constitution of Diets

Three diets including a standard control (*Pangran*), a control (*Panmor*) and an experimental (*Paneuph*) were defined. These diets were composed as follows:

- *Pangran* (diet control), composed of *Panicum maximum* and FACI mixed type rabbit pellet.
- *Paneuph*, composed of *Panicum maximum* and FACI mixed type rabbit pellet and *Euphorbia heterophylla*.
- *Panmor*, composed of *Panicum maximum* FACI mixed type rabbit pellet and *Moringa oleifera*.

The quantities of food distributed varied according to dry matter content and diets, which were otherwise iso-energetic and iso-proteic. They also varied from the adaptation period to the gestation period. However, they remained constant from gestation to lactation. *Panicum maximum*, *Euphorbia heterophylla*, and *Moringa oleifera* fodders were harvested in mid-day and sanitized with Topjavel© 2.4% active chlorine bleach (calcium hypochlorite). For this purpose, the fodder was immersed in 10 L of water containing 2 ml of bleach, then removed and dehydrated at room temperature on a permeable cloth for 12 h before being distributed to the animals.



Fig 3: Maternity cage

3.1.2 Conduct of the Test

The test lasted 58 days divided into 2 periods: (i) gestation period (30 days), and (ii) lactation period (28 days). These two periods were preceded by a 25-day adaptation period to allow the female rabbits to adapt to the breeding conditions. This adaptation period started with the distribution of the 18 female rabbits in the hutch cages and their random allocation of a diet. The cages were then numbered from 1 to 6, preceded by the initial feeding of test fodder for the experimental diets or T for the control diet. The numbering of the cages was as follows:

Rabbits on the control diet: T1, T2, T3, T4, T5 and T6;

Rabbits under the *Paneuph* regime: E1, E2, E3, E4, E5 and E6;

Rabbits under the *Panmor* regime: M1, M2, M3, M4, M5 and M6;

The adaptation period also made it possible to determine the average intake levels of the female rabbits in order to better estimate the amount of food to be distributed during gestation and lactation.

During this period, bromatological analyses of different samples of all foods used during the test were performed according to the standards [15].

The gestation period was marked by the natural mating of female rabbits with locally bred males from INP-HB production farm over a period of three (3) days. All the female rabbits were weighed before mating. Thus, the mating weight of each animal was recorded. The animals were weighed at the end of weeks 3 and 4 of the gestation period to assess their body condition and to confirm their pregnancy status.

3.2 Lactation period

As for the lactation period, it started at the farrowing of the rabbits. After controlling the fertility rate and the litter size, these litters were balanced at six bunnies per female in all diets. During the lactation phase, the female rabbits were weighed on an empty stomach every 7 days precisely at 7:30 a.m. to assess the evolution of their body condition. The bunnies were weighed on days 15 and 21, which corresponds to the peak of lactation [16].

3.3 Evaluation of Milk Production

The evaluation of milk production during the peak period consisted of determining the weight loss of the female rabbits during each feeding on the 15th and 21st day of the lactation period. To do this, the female rabbits were isolated from their litters the day before the weighing day at 6:00 p.m., and the next day at 7:30 a.m., they were weighed before being allowed to return to their nest boxes to nurse the bunnies. After 10 to 15 minutes of feeding, the females were removed and reweighed. The difference in weight between the two weighings corresponded to the amount of milk produced by the female rabbit [17].

Throughout the test period, feeding of each experimental diet was done manually and twice a day (8:00 a.m. for fodders and 5:00 p.m. for pellets). All the foods were weighed before being distributed to the animals. Every morning, the refusals were collected and weighed in order to evaluate the level of voluntary daily intake of dry matter (VDIDM) and the determination of the dry matter (DM) of the refusals. The weekly weighing of the female rabbits allowed evaluating the evolution of their body condition.

3.4 Data Analysis

The zootechnical parameters measured were voluntary daily intake of dry matter (VDIDM), average daily gain (ADG) of female rabbits, number of mated females, weight of mated female rabbits, number of females having given birth, weight of female rabbits at birth, average litter size, average weight of bunnies at birth, fertility rate, milk production at peak lactation of female rabbits, average weight and average daily gain of bunnies at peak lactation. At the end of the study, VDIDM, ADG and milk production of the female rabbits, the average weight and ADG of the bunnies at peak lactation, and the fertility of the female rabbits were determined according to the following formulas:

Voluntary Daily Intake of Dry Matter (VDIDM)

The Voluntary Daily Intake of Dry Matter (VDIDM) was determined from the amount of fresh matter ingested and was calculated as follows:

- $VDIDM = \text{amount of fresh matter ingested} \times \text{dry matter rate of the food ingested}$.

Fresh matter intake = quantity distributed - quantity refused

- ADG of female rabbits and bunnies

Measurements of the average daily gain of female reproducers and their bunnies were carried out according to the following formula:

AWi: average initial weight per cage

AWd: average weight on the first day of lactation.

d-i: Number of days

Growth parameters of Bunnies

The different weights collected allowed obtaining the average weight of the bunnies from the same litter according to the following formula:

$$\text{Average weight (g)} = \frac{\text{Total weight of the bunnies}}{\text{Number of bunnies}}$$

Fertility rate

$$\text{Fertility rate} = \frac{\text{Number of females having given birth}}{\text{Number of mated females}} \times 100$$

Bromatological analysis

The bromatological analyses included the determination of dry matter, ash, fatty matter, cellulose and nitrogen content of the various samples by the oven drying, muffle furnace calcination methods [18, 19, 20]. For this purpose, three samples of each food were used for the different analyses.

2.5 Statistical analyses

Calculations of the VDIDM, the quantity of milk produced at the peak of lactation of female rabbits, and the ADG of female rabbits and bunnies were performed using MICROSOFT Excel 2013 software. All statistical analyses were performed using XLSTAT version 2014. The multiple comparisons of the averages were performed at the 5% significance level by the Student-Newman-Keuls test (homogeneous variables) or by the non-parametric Kruskal-Wallis test (non-homogeneous variables). The difference between the averages is considered significant for $P < 0.05$.

3 Results and Discussion

3.1 Results

3.1.1 Nutritional Value of Foods Used

Table I presents the results of the bromatological analysis of the foods used during the test. These results show that the rabbit pellet had the highest levels of dry matter (expressed as % of fresh matter) and in metabolizable energy. These values were $90 \pm 0.4\%$ and $10.2 \pm 0.1\%$ for DM and ME contents respectively. The DM content of the pellet was three times higher than that of the other foods.

The organic matter (OM), mineral matter (MM) and fatty matter (FM) contents were approximately equal for all foods used. These values were 90%; 10% and 2.5% for the contents of OM, MM and FM respectively.

Moringa oleifera had the highest levels of crude fibre (CF) and total nitrogen matter (TNM). These values were $24 \pm 0.6\%$ and $22.7 \pm 0.5\%$ for CF and TNM contents respectively.

3.1.2 Quantity of food distributed during the test

Table II shows the quantities of food in fresh and dry matters distributed during the test. This table also shows the quantities of energy provided by each food, as well as the energy provided by each of the three formulated diets. The diets were formulated on the basis of the quantity ingested by the female rabbits during the adaptation period and the energy level of the different diets.

All the formulated diets provided the same quantity of energy (300 Kcal).

Regarding the quantities of dry matter, the *Panmor* diet had a slightly higher content (138.16 g. d-1.DM), followed by the *Paneuph* diet (133.49 g. d-1.DM), and the *Pangran* diet (126.57 g. d-1.DM).

3.1.3 Zootechnical parameters

3.1.3.1 VDIDM and ADG of female rabbits during gestation period

Voluntary daily intakes of dry matter of female rabbits measured during the gestation period were 102.5 ± 18.1 , 104.5 ± 25.8 and 116.6 ± 17.4 g. d-1.DM for the *Paneuph*, *Pangran* and *Panmor* diets respectively (Table III). The VDIDM of female rabbits on the different diets were significantly different ($P < 0.05$).

The intake levels of the different foods composing the diets were not compared because the quantities of dry matter distributed from the different foods (*Panicum maximum*, *Euphorbia heterophylla*, *Moringa oleifera*, *FACI pellet*) were not identical.

The ingestion of these diets induced ADGs over the entire gestation period of 9.6 ± 3.3 ; 11.7 ± 5.8 and 10.3 ± 3.8 g.d-1 for females on the *Paneuph*, *Pangran* and *Panmor* diets respectively (Table III). These ADGs were not significantly different ($P > 0.05$).

3.1.3.2 VDIDM and ADG of female rabbits during the lactation period

Over the lactation period, the average values of VDIDM for the experimental diets were 119.4 ± 9.1 ; 121.1 ± 5.8 and 131.8 ± 7.7 g. d-1.DM for *Paneuph*, *Pangran* and *Panmor* respectively (Table III). The VDIDM of female rabbits on the different diets was significantly different ($P < 0.05$).

The intake levels of the different foods composing the diets were not compared because the quantities of dry matter distributed from the different foods (*Panicum maximum*, *Euphorbia heterophylla*, *Moringa oleifera*, *FACI pellet*) were not identical.

The ingestion of these diets induced ADGs that were $-14.7 \pm$

2.6; -13.5 ± 3.4 and -15.4 ± 3.6 g. d⁻¹ for females on the *Paneuph*, *Pangran* and *Panmor* diets, respectively (Table III). The ADGs of female rabbits during the lactation period showed no significant difference ($P>0.05$).

3.1.3.3 Production and Reproduction Parameters of Female Rabbits

Table IV presents the production and reproduction parameters.

At farrowing, the weights of the female rabbits were 2.9 ± 2.5 , 2.9 ± 1.7 and 3.0 ± 6.7 kg for female rabbits on the *Paneuph*, *Pangran* and *Panmor* diets, respectively. The weight of female rabbits at farrowing showed no significant difference ($P>0.05$).

At the level of fertility rates, female rabbits on the *Pangran* diet were less fertile (66.6%) compared to rabbits on the *Paneuph* and *Panmor* diets, both of which had a fertility rate of 83.3%.

Concerning the number of live-born bunnies, the *Paneuph*

and *Panmor* diets recorded more bunnies (28 bunnies/diet) than the *Pangran* diet (24 bunnies).

The analysis of average litter sizes and average weights of bunnies at birth and weaning showed that there was no significant difference in either of these parameters ($P>0.05$). With the exception of female rabbits on the *Pangran* diet, the quantities of milk produced during the peak period were not significantly different. Indeed, female rabbits on the *Pangran* diet produced significantly less milk than female rabbits on the other two diets ($P<0.05$).

The values of average weights of bunnies at peak lactation and at weaning are presented in Table IV. These values showed no significant difference at these two periods. The ADGs at peak lactation were 6.9 ± 2.0 g d⁻¹, 6.6 ± 1.4 g d⁻¹, 6.3 ± 1.2 g d⁻¹ and 5.8 ± 1.1 g d⁻¹ for bunnies on *Paneuph*, *Pangran* and *Panmor* respectively (Table IV). The values of ADGs of bunnies at peak lactation and during the overall lactation period did not differ significantly ($P>0.05$).

Table 1: Nutrient composition of foods used during the test

Chemical composition (% DM)	Experimental foods			
	<i>Euphorbia heterophylla</i>	Rabbit pellet (FACI)	<i>Moringa oleifera</i>	<i>Panicum maximum</i>
Dry matter*	17,4 ± 0,2	90 ± 0,4	13,3 ± 0,6	25,8 ± 0,5
Organic matter	91,8 ± 0,6	90,1 ± 0,4	88,6 ± 0,5	90,7 ± 0,3
Mineral matter	8,2 ± 0,2	9,6 ± 0,4	11,4 ± 0,3	9,3 ± 0,5
Crude fibre	17,1 ± 0,5	14,6 ± 0,3	24,0 ± 0,6	17,4 ± 0,4
Total nitrogen matter	16,5 ± 0,2	16 ± 0,3	22,7 ± 0,5	10,2 ± 0,1
Fatty matter	2,31 ± 0,2	3,3 ± 0,1	2,8 ± 0,1	2,0 ± 0,2
Metabolisable energy Md.kg ⁻¹ DM *	9,2 ± 0,1	10,2 ± 0,1	6,3 ± 0,9	8,9 ± 0,1

Table 2: Quantities distributed in fresh matter, dry matter and provided energy

Diets	Experimental foods	Quantity of distributed food		Energy provided per food (Kcal)	Total food per diet (g.DM)	Total energy per diet (Kcal)
		Fresh matter (g)	Dry matter (g.DM)			
Pangran	<i>Panicum</i>	116,14	30	64,01	126,57	300,01
	Granulated	107,3	96,57	236		
Paneuph	<i>Panicum</i>	116,14	30	64,01	133,49	300
	Granulated	30,17	27,15	66,36		
	<i>Euphorbia</i>	438,22	76,34	169,64		
Panmor	<i>Panicum</i>	232,29	60	128,02	138,16	300,01
	Granulated	64,2	57,78	141,20		
	<i>Moringa</i>	152,86	20,38	30,78		

Table IV: Zootechnical Parameters

Parameters	Experimental Diets		
	Paneuph	Pangran	Panmor
Number of mated females (female rabbits)	6	6	6
Weight of mated female rabbits (g)	3030,0 ± 264,2	3006,3 ± 232,5	2999,0 ± 140,9
Number of females having given birth (female rabbits)	5	4	5
Weight of female rabbits at farrowing (g)	2957,8 ± 259,1	2966,9 ± 174,2	3032,4 ± 67,3
Average litter size (bunnies)	5,6 ± 1,1	6,0 ± 1,8	5,6 ± 2,2
Average weight of bunnies at birth (g)	54,3 ± 12,7	50,2 ± 8,9	56,1 ± 11,3
Fertility rate (%)	83,3	66,6	83,3
Milk Production of female rabbits (g)	140,0 ± 31,4 ^a	109,6 ± 22,0 ^b	131,5 ± 29,6 ^a
Average weight of bunnies (g)	192,0 ± 22,0	187,4 ± 35,1	185,6 ± 18,6
ADG of bunnies (g.d ⁻¹)	6,6 ± 1,4	6,3 ± 1,2	5,8 ± 1,1

Table 3: VIDIM and ADG of female rabbits during gestation and lactation

Physiological stages	Parameters	Experimental diets							
		Paneuph			Pangran		Panmor		
		102,5 ± 18,1 ^b			104,5 ± 25,8 ^b		116,6 ± 17,4 ^a		
Gestation	VIDIM (g.DM)	Pan	Euph	Gran	Pan	Gran	Pan	Mor	Gran
		23,0 ± 6,4	50,2 ± 16,4	24,4 ± 2,6	23,1 ± 5,5	81,7 ± 24,3	39,9 ± 9,2	21,3 ± 6,8	50,4 ± 7,2

	ADG of female rabbits (g.d ⁻¹)	9,6 ± 3,3			11,7 ± 5,8		10,3 ± 3,8		
Lactation	VDIDM (g.DM)	Paneuph			Pangran		Panmor		
		121,0 ± 6,2 ^b			121,5 ± 5,3 ^b		132,8 ± 7,5 ^a		
		Pan	Euph	Gran	Pan	Gran	Pan	Mor	Gran
	23,4 ± 3,2	70,6 ± 4,3	27,0 ± 0,0	25,5 ± 3,5	96,0 ± 2,9	46,3 ± 5,7	29,0 ± 3,6	57,6 ± 0,0	
	ADG of female rabbits (g.d ⁻¹)	-14,7 ± 2,6			-13,5 ± 3,4		-15,4 ± 3,6		

4. Discussion

The significant differences observed in the voluntary daily intake of dry matter in the diets used in the test would be related, on the one hand, to variations in the quantities of the granulated food in the diets and, on the other hand, to the nature of the foods.

The higher intake of the *Panmor* diet (124.7 ± 12.45 g. d-1.DM) compared to the *Paneuph* diet (111.75 ± 12.15 g. d-1.DM) would be explained by the relatively high quantities of the granulated food in the *Panmor* diet (64.2 g) compared to the *Paneuph* diet (30.17 g). This difference between the intake levels of the *Paneuph* and *Panmor* diets can also be explained by the high intake of *Euphorbia heterophylla* (60.4 ± 11.3 g. d-1.DM) which would be due to its organoleptic quality compared to *Moringa oleifera* (21.3 ± 6.8 g. d-1.DM). In fact, granulated rabbit food is highly appreciated by rabbits due to its compact and dry form [21], the fine particle size due to the grinding process and the nutritional values (minerals, vitamins and amino acids) of the raw materials used, such as soybean meal, which is used as a source of protein directly assimilable by the rabbit organism. Thus, when the rabbit has a choice, it benefits more from it [22]. Thus, the low level of rabbit pellets in the *Paneuph* diet will lead the rabbit to ingest a large quantity of *Euphorbia heterophylla* to make up for its energy deficit. However, since the animal's ability to ingest fresh material is limited, it will not be able to ingest a large quantity of this fodder compared to dry (pelleted) feed. The inefficiency of green fodder lies, among other things, in the quantity of energy withdrawn by the animal, which is fundamentally limited in fodders due to congestion [22].

The low level of intake of the *Pangran* diet compared to the *Panmor* diet would be related to rationing due to the excessively high energy level of the pellet (10224.9 ± 172.8 KJ.kg⁻¹MS) compared to other foods. As this diet is based on pellets, the quantities of food distributed were reduced in order to obtain iso-energy and isoprotein diets. Therefore, due to its digestive physiology, the rabbit consumes less *Panicum maxima* compared to the granulated diet. Indeed, rabbits selectively retain fluids and fine digesta particles during transit through the large intestine thanks to the muscular contractions of the walls of the colon which separate the particles according to their size. Coarse (more fibrous) particles are excreted faster than finer particles and in solution [22].

Voluntary daily intake of dry matter levels of the registered diets are lower than those obtained by Kouakou *et al.* (2016) [22], who recorded a VDIDM of 236.5 ± 11.3 g DM and 194.1 ± 17.3 g DM for rabbits on *Pangran* (granulated diet for rabbits and *Panicum maximum*) and *Paneuph* (diet composed of *Panicum maximum* and *Euphorbia heterophylla*), respectively. This difference could be due to the quantities of food available to the animals. Indeed, the "raw" ingestion capacity of rabbits for whole green plants is high, up to 40% of their live weight. On the other hand, the regulation of dry matter intake is relatively constant, ranging between 70 and 80 g/d/kg of metabolic weight given by the

formula AW = live weight 0.75 [23].

The better milk production of female rabbits under the *Paneuph* and *Panmor* experimental diets is due to the galactogenic action of *Euphorbia heterophylla* and *Moringa oleifera* brought to the diets. The galactogenic activity of these plants would be due to their ability to stimulate the secretion of hormones that promote milk synthesis, particularly prolactin [24]. Indeed *Euphorbia heterophylla* and the capsules of the leaves of *Moringa oleifera* contain chemical compounds of phytosterol (poliferol and sterols), saponins, phenols and terpenes including phorbolic diterpenes playing a role in increasing prolactin levels [25, 13]. The daily quantities of milk obtained in female rabbits under the *Paneuph* and *Panmor* experimental diets are lower than those obtained by Rekik and Bergaoui (2016) [16] in female rabbits of crossbred "New Zealand X Californian X local (Tunisia)" strains. These authors obtained 220 g.d⁻¹ with a diet composed of 15.5% protein, 4% crude fat, 15.5% crude fibre, 2,600 kcal DE and 2% fenugreek. As the nutritional compositions of the foods used in these two tests were approximately identical, this difference in milk cannot be related to diet. However, it would probably be due to litter size. These authors used seven versus six bunnies in our test. Indeed, according to Chibah-Ait Bouziad *et al.* (2015) [26], milk production of female rabbits is important when the size of the nursing litter is large.

The negative average daily gains observed at the end of lactation in this experiment would be due to the extremely energy-intensive function of milk production. Indeed, female rabbits do not ingest a sufficient quantity of food to cover their milk production and maintenance needs [27]. This intense nutritional demand results in high body mobilization [28, 29]. These results are in agreement with those observed by Kouakou *et al.* (2016) [22] with local female rabbits raised under the same conditions with an average ADG at the end of the lactation period of -10.7 ± 3.0 g.d⁻¹.

The average daily gains of bunnies during the peak lactation period under the three diets were not significantly different despite the better milk production of female rabbits under the experimental diets. This growth performance of the bunnies would probably be related to the quality of the different milks.

Based on the ADGs of bunnies, the milk of female rabbits on the control diet seems to be richer in nutrients such as calcium, magnesium, phosphorus, zinc, etc. than the milk of female rabbits on the experimental diets. However, only the analysis of the nutritional quality of the different milks would confirm these results. Under these conditions, it is undeniably important to deepen the studies on the nutritional quality of the different milks obtained. According to Rekik and Bergaoui (2016) [16], the ingestion of milk that does not have all the desired nutritional characteristics would result in stunted growth. The ADGs obtained at the peak of lactation of 6.6 ± 1.4, 6.3 ± 1.2 and 5.8 ± 1.1 respectively for bunnies under *Paneuph*, *Pangran* and *Panmor* were lower than those of Kouakou *et al.* (2016) [22] who reported ADGs during the lactation period of 11.1 ±

4.7 and $8.4 \pm 2.5 \text{ g.d}^{-1}$ respectively for the *Pangran* and *Paneuph* diets with local bunnies from mothers twice as old. As the animals are of the same breed and reared under the same conditions, this difference between the ADGs would be related to the low milk production of the female rabbits due to their undernourishment of the diets applied in terms of quantity distributed. According to Lebas, (1969) ^[30], the growth of bunnies before weaning is conditioned by the female rabbit's milk production.

5. Conclusion

This study on the efficacy of the plants *Euphorbia heterophylla* and *Moringa oleifera* for milk production in female rabbits at peak lactation showed the positive effect of these plants on milk production. Indeed, interesting increases in milk production of the lots that received the experimental foods (*Euphorbia heterophylla* and *Moringa oleifera*) were noted in female rabbits. These foods also had a positive effect on the level of voluntary daily intake of dry matter, which resulted in a better intake of the *Panmor* diet. However, in view of the results obtained, it would be advisable to continue this work, with a view to detecting both the metabolites that induce this performance and their mode of action. Therefore, these results need to be confirmed on the one hand, by more precise measurements of milk quantity such as pre- and post-weighing of bunnies and on the other hand, by methods for the determination of hormones such as prolactin. However, it seems to us that the present work should be deepened by: setting up tests to determine the effect of *Euphorbia heterophylla* on the quality of the milk produced in order to allow the use of this plant in women who have difficulties in producing milk; the search for the hormones responsible for milk secretion through analyses of female rabbit blood plasma; the ethnobotanical study of *Euphorbia heterophylla* in order to understand its level of use at the national level.

6. References

- Djermoune S, et Henoune N. Composition chimique et teneur en composés phénoliques des graines de *Moringa oleifera*. Algérie: Université A. MIRA – Bejaia, 2015, 89 p.
- Natália Martins, Lillian Barros, Celestino Santos-Buelga, Mariana Henriques, Sónia Silva, Isabel C.F.R. Ferreira. Decoction, infusion and hydroalcoholic extract of *Origanum vulgare* L.: Different performances regarding bioactivity and phenolic compounds. *Food Chemistry*. 2014; 158:73-80.
- Cero MD, Saller R, et Wecker CS. The use of the local flora in Switzerland: A comparison of past and recent medicinal plant knowledge. *Ethnopharmacol*. 2014; 151:253-264.
- Arora DS, Onsare JG, et Kaur H. Bioprospecting of *Moringa* (Moringaceae): Microbiological Perspective. *J Pharmacogn. Phytochem*. 2009; 1:193-210.
- Muthu C, Ayyanar M, Raja N, et Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *Ignacimuthu*. 2006; 2:1-10.
- Baig MI, et Bhagwat VG. Study the efficacy of GALACTIN VET bolus on milk yield in dairy cows. *Vet World*. 2009; 2:140-142.
- Nancy G, Powers M, Anne M, et Montgomery. Utilisation des galactogènes pour l'induction ou l'augmentation de la sécrétion lactée. *Breastfeed. Med*. 2011; 1 :158-165.
- Belkadhi S. Tout savoir sur le *Moringa*. <https://lessecretsdesplantes.wordpress.com/2016/10/19/tout-savoir-sur-le-moringa>, 2016. Accessed on 18 septembre 2018.
- Maroyi A. *Calotropis procera* (Aiton) W.T.Aiton. *Prota Medicinal plants / Plantes médicinales*. 2012; 2:2012-2018.
- Adepo YP, Assi S, Biego HG, Chatigre KO, et Kati, CS. Étude de l'évolution des paramètres physico-chimiques de deux plantes *Euphorbia hirta* et *Secamone afzelii* en fonction des quatre saisons de l'année, de l'extraction aqueuse et évaluation du pouvoir lactogène. *Bull. Société R. Sci. Liège*. 2010; 79:12-24.
- Adépo YP, Bolou GEK, et Akoa EEF. Effet sur le développement mammaire et analyse phytochimique de deux plantes lactogènes de la pharmacopée traditionnelle ivoirienne: *Euphorbia hirta* L. et *Secamone afzelii* (Roem. &Schult.). *Int. J. Biol. Chem. Sci*. 2017; 11:1872-1877.
- Currasson MG. Sur quelques coutumes et légendes indigènes concernant la lactation http://remvt.cirad.fr/cd/EMVT49_4.PDF, 2018, 170-171.
- Mosango DM. *Euphorbia heterophylla* L. *Prota*. 2008; 11(1):1-2.
- Kouakou NDV, Grongnet JF, Assidjo NE, Thys E, Marnet PG, Catheline D, et al. Effect of a supplementation of *Euphorbia heterophylla* on nutritional meat quality of Guinea pig (*Cavia porcellus* L.). *Meat Sci*. 2013; 96:821-826.
- AOAC. Official Methods of Analysis of AOAC INTERNATIONAL 18th Edition, Current through Revision 1, 2005, p 18
- Rekik I, et Bergaoui R. Influence du fenugrec (*Trigonella fœnum græcum* L.) sur la production laitière des lapines et la croissance des lapereaux avant sevrage. *J New Sci*. 2016; 30:1709-1718.
- Lebas F. Mesure quantitative de la production laitière chez la lapine. *Annale de Zootechnie*. 1968; 17:169-182.
- Folch J, Lees M, Sloane-Stanley GH. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 1957; 226:497-509.
- Van Soest PJ, McQueen RW. The chemistry and estimation of fibre, *Proc. Nutr. SOC*. 1973; 32:123-130.
- Thompson M, Owen L, Wilkinson K, Wood R, Damant A. A comparison of the Kjeldahl and Dumas methods for the determination of protein in foods, using data from a proficiency testing scheme. 2002; 127:1666-1668.
- Kpodékon M, Youssao AKI, Koutinhoun GB, Baba IL, Dessou JM, et Djago Y, et al. Effet de la granulation sur les performances de croissance, l'efficacité alimentaire et la viabilité des lapereaux en condition d'élevage tropical. *Rev. Elev. Médecine Vét. Pays Trop*. 2009; 62:75-80.
- Kouakou NDV, Coulibaly SBM, Angbo-kouakou CEM, Thys E, Assidjo NE, et Kouba M, et al. Réduction des coûts alimentaires des lapins (*Oryctolagus cuniculus* L.) par la distribution de l'herbe de lait (*Euphorbia heterophylla* (L.) Klotz. & Garcke)

- associée à l'herbe de Guinée (*Panicum maximum* Jacq.) Lam. en élevage semi-intensif. J Appl. Biosci. 2016; 99:9373-9381.
23. Goby JP, Couillet S, Huck C, Bannelier C, et Gidenne T. Ingestion et digestion de fourrages secs et verts pour le lapin: Première approche pour la luzerne et la carotte entière. (Le Mans, France), 2017, 197-200.
 24. Akouededni CG, Adenile AD, Gbego IS, et Hounzangbe-Adote MS. Efficacité des feuilles de *Spondias mombin* L. sur la production laitière et la croissance pondérale des ovins djallonké au Sud-Benin. Ann. Sci. Agron. 2013; 17:137-148.
 25. Sulistiawati Y, Suwondo A, Hardjanti TS, Soejoenoes A, Anwar MC, et Susiloretni KA, *et al.* Effect of *Moringa oleifera* on level of prolactin and breast milk production in postpartum mothers. Belitung Nurs. J 2017; 3:126-133.
 26. Chibah-Ait Bouziad K, Zerrouki-daoudi N, et Lebas F. Effets de la taille de portée à la naissance et du nombre de lapereaux allaités sur les aptitudes laitières des lapines de deux génotypes. (Le Mans, France), 2015, 89-92.
 27. Feugier A. Une méthode alternative de reproduction chez la lapine: un modèle pour une approche systémique du fonctionnement des élevages cunicoles. Thèse doctorale. Institut National Polytechnique DE Toulouse, 2006, 157p.
 28. Fortun-Lamothe L. Energy balance and reproductive performance in rabbit does. Anim Reprod Sci. 2005; 93:1-15.
 29. Divya KG, Rubeena M, Andalil R, Erni B, Sundaramoorthy B, Kumar KN, Thatipelli S, Shakila R. Identity profile of *Moringa oleifera* Lam. Flower. Int J Bot Studies, 2019;4(4).
 30. Lebas F, et Sardi G. Alimentation lactée et croissance pondérale du lapin avant sevrage. Annales de zootechnie, INRA/EDP Sciences. 1969; 18(2):197-208.
 31. Pascual JJ. Early weaning of young rabbits. Rabbit Sci. 2001; 9:165-170.