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M. Kimsé, M.Y. Yapi, M. Karamoko, Thierry Gidenne, M. Zongo, B.I. Gnanda, A. Akoutey, N.C. Bodji, A. Fantodji, A. Otchoumou

World Rabbit Science

World Rabbit Sci. 2017, 25: 225-231
doi:10.4995/wrs.2017.5126

EFFECT OF TROPICAL GREEN FORAGE *PUERARIA PHASEOLOIDES* ADDITION TO A PELLETED COMPLETE FEED ON RABBIT GROWTH PERFORMANCE AND DIGESTION

KIMSÉ M.*, YAPI Y. M.†, KARAMOKO M.*, GIDENNE T.¶, ZONGO M.‡, GNANDA B.I.§, AKOUTEY A.#, BODJI N.C.†, FANTODJI A.*, OTCHOUMOU A.*

*Pôle Production Animale, Laboratoire de Biologie et de Cytologie Animale, UFR Sciences de la Nature, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.
†Laboratoire de Zootechnie et Productions Animales, DFR Agriculture et Ressources Animales, Institut National Polytechnique Félix Houphouët Boigny, BP 1093 Yamoussoukro, Côte d'Ivoire.
‡Laboratoire de Physiologie Animale, UFR Sciences de la Vie et de la Terre, Université de Ouagadougou, Ouagadougou, Burkina Faso.
§Institut de l'Environnement et de Recherches Agricoles (INERA), 04 BP 8645 Ouagadougou 04, Burkina Faso.
#Département de Production et Santé Animales de l'Ecole Polytechnique d'Abomey-Calavi, EPAC-UAC Cotonou, Bénin.
Laboratoire de Recherche en Biologie Appliquée (LARBA) de l'Ecole Polytechnique d'Abomey-Calavi, Cotonou, Bénin.
¶GenPhySE, Université de Toulouse, INRA, INPT, ENVT, F-31326 Castanet Tolosan, France.

Abstract: The aim of this work was to study the effect of tropical green forage on rabbit growth performance and apparent digestibility. Thirty rabbits weaned at 35 d of age were individually caged and allotted to 2 dietary treatments. From 35 to 90 d of age, the control group C was fed *ad libitum* with commercial pelleted diet C only, while the test group was fed the C diet and forage *Pueraria phaseoloides* (Pp) *ad libitum*. Individual water and feed intake, body weight gain, nutrient apparent digestibility, red and white blood cells were studied. Mean housing temperature was 27.7°C. Water intake (35-90 d) did not differ between the 2 groups (mean=128 mL/d), whereas feed intake (35-90 d) was twice as high, with Pp (114 *vs.* 56 g; P=0.02). Forage intake doubled every 2 wk, averaging 50% of the total intake from 35 to 90 d of age. The growth rate was higher (+30%) in the Pp group after weaning (35-49 d) but did not differ between groups thereafter. The feed conversion was higher for the Pp group after weaning only (+ 87%; P<0;05). Weight of rabbits and feed efficiency were not affected by forage addition. Organic matter digestibility of diet C alone was roughly twofold higher compared to C+Pp (P=0.03). Red blood cells were not affected by treatments (4.1×10^{12} cells/L). However, the white blood cell count was higher in Pp than in C group (7.4×10^{9} *vs.* 3.9×10^{9} cells/L; P<0.01). *P. phaseoloides* may be used as a complement to a balanced pelleted feed, but further studies with a large number of rabbits are necessary to analyse the potential impact on health status.

Key Words: blood cells, digestibility, growth, health, *Pueraria phaseoloides*, rabbit.

INTRODUCTION

Several forages are used in less developed countries by traditional rabbit farmers in order to reduce the feeding cost (Kimsé *et al.,* 2014). Hiep and Man (2008) showed, in Vietnam, that the basal diet of rabbits is generally based on roadside grass and agricultural products that are not suitable for other livestock and that the nutritive value of these feeds is low. Recent studies have shown that sun-dried *Pueraria phaseoloides* in diet did not affect rabbit feed intake (Akoutey *et al.,* 2012). Similar results were observed with other leguminous green forage. For example, young rabbit growth performance was not affected by the use of *Centrosema pubescens* as forage (Kimsé *et al.,* 2013), although the dietary crude protein level decreased (–20 g/kg dry matter [DM]) and fibre level increased (neutral detergent fibre [NDF]=+70 g/kg DM). Besides, *Syndrella nodiflora* forage increases daily gain (+5 g/d, Omoikhoje *et al.*, 2006). A 20% addition of *P. phaseoloides* in a diet increased daily gain (+3 g/d), but an inclusion at 40% decreased the

Correspondence: M. Kimsé, kimsemou_sn@una.edu.ci. Received *March 2016* - Accepted *March 2017.*
https://doi.org/10.4995/wrs.2017.5126

daily gain (–1 g/d, Akoutey and Kpodekon, 2012). *P. phaseoloides* forage has relatively high levels of crude protein (12 to 24% of the DM; Hiep *et al.,* 2008). In Côte d'Ivoire, *P. phaseoloides* is a sole cover crop which prevents soil erosion, stops weed and enriches the soil of latex, coconuts and oil-palm plantations. In traditional rabbit breeding system, breeders are using leguminous forage such as *C. pubescens* and *P. phaseoloides*, among others. However, few studies have tested the impact of this forage on rabbit performances and physiology. The aim of this work was to study the effect of green *P. phaseoloides* forage on rabbit growth performance, digestion and some physiological parameters.

MATERIAL AND METHODS

Animal housing conditions and diet

Thirty Ivorian patrimonial rabbits weaned at 35 d were divided in 2 groups. The average weaning weights of groups were equal (523 g).

Animals were housed indoors, in individual metabolism cages (52×40×40 cm) from weaning at 35 d of age to 90 d of age, and submitted to 12 h light (from 7:00 a.m. to 7:00 p.m. GMT) at 28°C. Animals had *ad libitum* access to water and diets (pellet and forage) throughout the test and were fed with 2 diets (Table 1). No antibiotics or any other drugs were added to the diet or drinking water. The control group was fed a commercial pelleted feed only (group C). The other group was fed the same commercial pelleted diet as in group C, supplemented with green *Pueraria phaseoloides* forage (group Pp). Forage was collected daily without considering its physiological state. It was collected and exposed for 24 h at 28°C before use. Fifty grams (50 g) of pelleted diet and 100 g of fresh forage material were sampled weekly to determine feed chemical composition. Room temperature was checked daily at 8:00 a.m. and 14:00 p.m.

Animal health, growth performance and feed intake

Rabbit live weight was individually recorded twice weekly from 35 to 90 d of age. Feed and water intake were recorded daily by weighing the diet and water dispensed and refused.

The number of morbid (diarrhoea) and dead rabbits was checked every morning before diet distribution. Animals whose weight decreased between two consecutive controls were considered morbid. The sanitary risk index (SRI) was calculated as the number of dead and morbid animals accumulated within a period.

Digestibility and chemical analysis

For digestibility measurement, rabbits were adapted to housing condition for 15 d. After the adaptation period, the hard faeces excreted daily were individually collected (n=15 healthy rabbits) for 4 consecutive days (50-53 d of age) to calculate the apparent faecal digestibility of nutrients (Perez *et al.,* 1995). Samples were stored at –20°C in plastic bags upon collection. Dry matter (DM), organic matter (OM), crude protein (CP) and fibre in feeds were analysed following the European Group on Rabbit Nutrition (EGRAN, 2001) recommendations. Analyses of detergent fibre

Table 1: Chemical composition (g/100 g dry matter) of the pelleted diet (diet C) and of the forage (*Pueraria phaseoloides,* Pp).

Nutrients	Diet C	Forage Pp
Dry matter (g/100 g)	85.7	49.9
Organic matter	91.0	92.7
Crude protein	8.5	9.0
Neutral-detergent fibre	43.4	47.2
Acid-detergent fibre	19.6	27.6
Celluloses	12.4	15.1

content (neutral degergent fibre [NDF], acid detergent fibre [ADF] and acid detergent lignin [ADL]) were performed using the sequential method of Van Soest *et al.* (1991).

Blood sampling and analysis

Blood was sampled in the morning, from marginal vein of ear at 50, 60 and 80 d of rabbit age. Venous blood was collected in a tube with anticoagulant ethylenediaminetetraacetic acid (EDTA). Red and white blood cell counts were quantified automatically using Sysmex PLC Xt 2000i.

Statistical analysis

The statistical analysis was performed using a monofactorial model (effect of diet: 2 levels). The effects of forage addition on growth, feed intake and feed apparent digestibility were treated by Student t-test. Effects of diet and age on blood cells were analysed using analysis of variance (ANOVA). These statistical analyses were performed using the R software version 3.0.2. Mortality, morbidity and sanitary risk index (SRI) were tested by Chi-square test with excel.

RESULTS

Feed and water intake and housing condition

During the trial, the rabbitry temperature averaged 27.7°C. The highest temperature was 28°C and the lowest was 26°C. Chemical composition of the diets is reported in Table 1. The DM was 2 times higher in pelleted diet than in forage. However, OM, CP and fibre contents were slightly higher in forage than in pelleted diet (+3, +5 and +4%, respectively). The average water intake was 128 mL/d rabbit from 35 to 90 d of age. No significant difference was found regarding water intake between the groups C and Pp (Table 2). The water intake increased logically with rabbit weight, according to the following relationship: water intake (mL)=0.1×Live weight (g)+40.55 (P<0.01, R^2=0.5). From weaning (35 d) to 90 d of age, the daily feed intake (g/d of DM) was twice as high in the Pp group as in the C group: 114 and 56 g/day respectively (P=0.02). For the first 2 wk (from 35 to 49 d of age), feed intake was similar in the control and Pp groups (38.6 g/d). However, between 50-63 d, feed intake was 35 g higher in the Pp group than in control. From 64 to 77 and 78 to 90 d of age and over the whole fattening period (35-90 d), ingestion was twice as high in the Pp group as in the control group. The quantity of forage ingested doubled every 2 wk. The forage part represented 50% of diet intake from 35 to 90 d of age.

Table 2: Effect of forage addition (*P. phaseoloides*) to a pelleted diet on feed and water intake of the growing rabbit.

	Diet C	Diet Pp	RSE	*P*-value
Feed intake (g/d)[1]				
35-49 d	30	47 (18)	19.9	0.16
50-63 d	52	87 (36)	12.7	<0.01
64-77 d	70	153 (87)	20.2	<0.01
78-90 d	72	168 (83)	38.9	0.06
35-90 d	56	114 (56)	18.2	0.02
Water intake (mL/d)				
35-49 d	109	88	18.0	0.22
50-63 d	120	111	31.3	0.73
64-77 d	144	134	44.6	0.80
78-90 d	140	119	24.0	0.33
35-90 d	128	113	31.1	0.24

RSE: residual standard error. C: Control group fed with commercial pelleted diet; Pp: Group fed forage (*Pueraria phaseoloides)* and a commercial pelleted diet; [1] data in brackets correspond to forage intake (*Pueraria phaseoloides*).

Table 3: Effect of forage addition (*Pueraria phaseoloides*) to pelleted diet on growth performance and feed conversion of the rabbit.

	Diet C	Diet Pp		
Number	15	15	RSE	*P*-value
Weight (g)				
Age (days)				
35 d	534	534	116.7	0.90
49 d	631	690	189.2	0.21
63 d	902	971	195.0	0.22
77 d	1199	1296	204.1	0.26
90 d	1474	1595	234.8	0.30
Daily weight gain (g/d)				
Age (days)				
35-49 d	6.9	11.2	5.5	0.05
50-63 d	19.4	20.1	3.78	0.49
64-77 d	21.2	23.2	5.35	0.57
78-90 d	21.2	23.0	2.81	0.53
35-90 d	17.1	19.3	2.81	0.51
Feed conversion ratio				
Age (days)				
35-49 d	4.3	4.2	0.29	0.10
50-63 d	2.3	4.3	0.10	<0.01
64-77 d	3.3	6.6	0.63	0.28
78-90 d	3.4	7.3	1.70	0.16
35-90 d	3.3	5.8	0.58	0.33

RSE: Residual Standard Error. C: Control group feed with commercial pelleted diet; Pp: Group feed with *Pueraria phaseoloides* and commercial pelleted diet.

Growing performances and digestibility

Forage addition did not affect the growth rate (Table 3) over the whole fattening period (35-90 d), which averaged 18 g/d. However, from 35 d to 49 d the average daily weight gain of Pp group was 4 g higher than in control (P=0.05). Feed conversion ratio was not different between the 2 groups except from 50 to 63 d (P<0.01). From 50 to 63 d of age, feed conversion was 2 units higher with Pp compared to control (P<0.01). From weaning to slaughter, feed conversion was similar (4.6) between the 2 groups.

Table 4: Effect of forage addition (*Pueraria phaseoloides*) to a pelleted feed on nutrient digestibility (%).

	Diet C	Diet Pp	RSE	*P*-value
dDM	47.8	18.0	10.09	0.03
dMO	51.8	24.2	9.18	0.03
dCP	78.9	72.3	5.83	0.43
dNDF	26.6	44.5	9.16	0.12
dADF	10.9	29.3	10.46	0.07
dHC	39.5	65.8	14.19	0.14

RSE: Residual Standard Error. C: Control group fed with commercial pelleted diet; Pp: Group fed with *Pueraria phaseoloides* and commercial pelleted diet. dDM: digestibility of dry matter; dMO: digestibility of organic matter; dCP: digestibility of crude protein; dNDF: digestibility of neutral detergent fibre; dADF: digestibility of acid detergent fibre; dHC: digestibility of hemicellulose.

Digestibility of the dry matter and organic matter (Table 4) was 2.7 and 2.1 times higher respectively for control than for forage group (P=0.03). Reversely, ADF digestibility of Pp was 2.7 times higher that of control (P=0.07). However, CP, NDF and hemicellulose digestibility were not different between C and Pp.

Blood cell rates

There was no interaction between effect of diet and age on rabbit red and white blood cells (Table 5). Red blood cell count was not affected by diet, and averaged 4.1×10^{12} cell/L. However, from 50 to 60 d of age, red blood cell concentration decreased from 5.0×10^{12} cell/L to 2.4×10^{12} cell/L (P<0.01). The cell count was no different at 50 d and 80 d. The average number of red cells at 50^{th} and 80^{th} days of age was 5×10^{12} cell/L.

Table 5: Effect of forage addition (*Pueraria phaseoloides*) to pelleted diet and age on rabbit red and white blood cells.

	Diet		Age (days)			RSE	*P*-value		
	C	Pp	50 d	60 d	80 d		Diet	Age	Diet×Age
No. of rabbits	12	12	8	8	8				
Red blood cells (×10^{12}/L)	4.1	4.0	5.0^{b}	2.4^{a}	4.9^{b}	0.58	0.35	<0.01	0.77
White blood cell (×10^{9}/L)	3.9	7.4	7.00	5.4	4.2	2.63	<0.01	0.30	0.24

^{a,b}Means in a row and effect not sharing superscript are significantly different at *P*<0.05; RSE: Residual standard error; C: Control group feed with commercial pelleted diet; Pp: Group feed with *Pueraria phaseoloides* and commercial pelleted diet.

The number of white blood cells was twice as high in the *P. phaseoloides* group as in the control (*P*<0.01). However, there was no significant effect of age on the white blood cell count between 50 d and 80 d of age. The average concentration of white blood cells during the experiment was 5.51×10^{9} cell/L.

Health status

The health status according to age and diet is shown in Table 6. Diarrhoea incidences from 35 to 90 d of age were observed, due to a colibacillosis outbreak with profuse diarrhoea symptoms. However, as only 15 rabbits per group were used, significant differences for mortality or morbidity rate were hardly detectable. Nevertheless, we provide some results to indicate the trends observed. Mortality tended to be higher in Pp group than in C group, but this result needs to be confirmed. From weaning to 49 d of age, 8 rabbits were morbid in the Pp group compared to only 1 rabbit in the control group (*P*<0.01). SRI was thus higher in the Pp group than in the control group from weaning to 49 d of age (*P*<0.01), and tended to be higher from 50 to 63 d of age (*P*=0.07). However, SRI was similar in the 2 groups over the whole trial (35 to 90 d).

Table 6: Effect of forage addition (*Pueraria phaseoloides*) to a pelleted diet on animal health from 35 to 90 d.

	Diet C	Diet Pp	*P*-value
35-49 d			
Initial number	15	15	
Mortality	1	3	0.28
Morbidity	1	8	<0.01
SRI	2	11	<0.01
50-63 d			
Initial number	14	12	
Mortality	2	5	0.12
Morbidity	5	5	0.76
SRI	7	10	0.07
64-77 d			
Initial number	12	7	
Mortality	0	1	0.18
Morbidity	5	1	0.22
SRI	5	2	0.57
78-90 d			
Initial number	12	6	
Mortality	2	1	1
Morbidity	4	2	1
SRI	6	3	1
35-90 d			
Initial number	15	15	
Mortality	5	10	0.07
Morbidity	5	1	0.07
SRI	10	11	0.69

SRI: Sanitary Risk Index, C: Control group feed with commercial pelleted diet; Pp: Group feed with *Pueraria phaseoloides* and commercial pelleted diet.

DISCUSSION

In this study, protein rates in the forage and control diets were lower than recommended in rabbit nutrition (Martignon *et al.,* 2010; Akoutey *et al.,* 2012; Kimsé *et al.,* 2012). Nevertheless, feed intake doubled with the addition of *P. phaseoloides*. The effect of forage addition on feed intake has been reported by several studies. For instance, Kimsé *et al.* (2013) and Omoikhoje *et al.* (2006) also observed a higher intake when forage was added to the pelleted diet. Forage effect on rabbit ingestion varies with the type of plant. Soybean substitution by *P. phaseoloides* foliage (20%) increased feed intake (Hiep *et al.,* 2008). When animals are fed with forage, their caecum volume increases (Jenkins, 1999). This physiological aspect coupled with the high fibre level in forage diet could explain the high ingestion observed in rabbits fed forage. Feed intake is also influenced by the palatability and physical and chemical characteristics of the diet, as well as its presentation format. Fat incorporation in the diets, while maintaining the dietary fibre level, increases the dietary digestible energy level and slightly reduces ingestion (Gidenne *et al.,* 2010). Other nutrients such as protein and amino-acids can modify feed intake (Tome, 2004), and high temperatures and feed presentation are both able to lower feed intake (Gidenne *et al.,* 2010). This may explain the low consumption of rabbits reared in Côte d'Ivoire compared to those in Europe.

Growth performance was not affected by the high forage ingestion, except for the daily gain during the first 2 wk after weaning. Daily gain and feed efficiency observed in this study are similar to previous studies on the use of forage in the diet of rabbit reared in tropical conditions (Hiep and Man, 2008; Akoutey *et al.,* 2012; Kimsé *et al.,* 2013), but higher than the values observed by Omoikhoje *et al.* (2006). Organic matter digestion of the diet dropped when forage was added. Similar results were reported by Akoutey *et al.* (2012). Conversely, the better fibre digestion observed with Pp would suggest that the cell wall polysaccharides contained in the whole *Pueraria* were highly digestible.

Between 50 and 63 d of age, the red blood cell concentration was low and may suggest a possible anaemia of the animals (Blood and Studdert, 1999). However, the white blood cell count remained unchanged with age, but increased with forage addition. Similar results have been shown using *P. phaseoloides* green forage and *Syndrella nodiflora* (Omoikhoje *et al.,* 2006; Bleyere *et al.,* 2013).

CONCLUSION

The use of *Pueraria phaseoloides* green forage as a complement to complete pelleted feed in rabbit feeding decreased the intake of pelleted diet with no detrimental effect on growth performance. Thus, green *P. phaseoloides* may be used to complement pelleted diet in traditional breeding systems. Studies on a large number of rabbits are needed to better assess the impact of green *P. phaseoloides* on growth and health performance of rabbits reared in tropical conditions. Further studies should include the total composition of nutrients ingested in forage and pellets. These parameters will help better explain the relation between intake, growth and health.

Acknowledgement: Authors would like to thank: Bléyéré N.M, Kadjo V., Outtara A., Yapi J.N., Coulibaly K.A.S of Université Nangui Abrogoua for taking care of the animals and for providing technical assistance.

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