

Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 15(3): 869-878, June 2021

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper

http://ajol.info/index.php/ijbcs

http://indexmedicus.afro.who.int

Effects of incorporation levels of *Pueraria phaseoloides* leaf flour on carcass characteristics and chemical composition of meat from local rabbit (*Oryctolagus cuniculus*) in South-East Gabon

Mamadou THIAM¹, Gilbert Comlan ZOUGOU TOVIGNON^{1*}, Abdourhamane Ibrahim TOURE¹, Moussa KIMSE², Cédric SIMA OBIANG⁴, Arsène Valery MBOKO¹, Férence Ndzani MATUMUINI¹, Frédéric Christol NGANSOP NONO³, Louis Clément OBAME ENGONGA⁴, Joseph-Privat ONDO⁴, Fernand TENDONKENG⁵, Emile MIEGOUE⁵, Benoit BOUKILA¹ and Etienne PAMO TEDONKENG⁵

¹ National Higher Institute of Agronomy and Biotechnology (INSAB), University of Science and Techniques Masuku (USTM), P.O. Box 941 Franceville, Gabon.

² Animal Production Pole, UFR Sciences of Nature, Nangui Abrogoua University, 02 P.O. Box 801 Abidjan 02, Côte d'Ivoire.

³ Technical School of Agriculture (TSA) Bambili, P.O. Box 15, Bambili (Bamenda), Cameroon. ⁴ Laboratory of Biochemistry, Faculty of Science of the University of Science and Techniques

Masuku (USTM), P.O. Box 941 Franceville, Gabon.

⁵ Laboratory of Animal Nutrition, Department of Animal Science, FASA, University of Dschang, P.O. Box 222 Dschang, Cameroon.

*Corresponding author; E-mail: zougou.g@gmail.com

Received: 15-01-2021 Accepted: 22-06-2021 Published: 30-06-2021

ABSTRACT

The objective of this study was to contribute in improving knowledge on rabbit feeding in Gabon. This work was conducted with the aim of studying carcass characteristics and chemical composition of meat from rabbit induced by feeds containing *P. phaseoloides*. To that effect, 20 young female rabbits from local breed, weighing averagely 611 ± 33.20 g and aged about 6 weeks were randomly distributed in 4 groups of 5 animals each. The groups R0, R15, R20 and R25 were fed rations containing respectively 0%, 15%, 20% and 25% of *P. phaseoloides*. At the end of the trial, animals were slaughtered then eviscerated in order to evaluate carcass characteristics. Muscles were taken, ground and mixed for chemical analysis. No matter the ration, carcass characteristics showed no significant difference (p > 0.05). Highest protein contents in meat were recorded in animals from R15 (23.90% DM) and R20 (22.46% DM). The lowest fat content (5.41% DM) was that of animals from R25, followed by that from R20 (6.67% DM). Based on these results obtained, the ration containing 20% *Pueraria phaseolides* (R20) could be recommended.

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Keywords: Rabbit, Pueraria phaseoloides, carcass, meat, chemical composition, South-East Gabon.

INTRODUCTION

Food insecurity constitutes a major problem in Africa taking into consideration the imbalance between food availability and population growth (PNUD, 2012). The development of rabbit production (cuniculture) could contribute in the reduction of hunger and protein malnutrition in populations (Kouakou et al., 2016). Rabbit (Oryctolagus cuniculus) is a highly prolific species with a rapid growth. Its flesh is tender, tasty and nutritive and it presents dietary qualities with less fats and cholesterol (Djago et al., 2007). However, cuniculture faces many constraints among which there is improper feeding. One of the most important factors for the success and the economy of this livestock lies in feeding (Dahouda et al., 2013). Feeding represents 70% of total production charges and the reduction of feed costs becomes an important preoccupation for animal keepers (Defang et al., 2014). Conventional protein sources become more and more scarce and expensive (Braine, 2008). In front of this situation, research on the valorization of alternative feed resources locally available could permit to improve the productivity of animals while mastering the costs of inputs (Ouédraogo et al., 2015). Many studies have been carried out on the incorporation of non-conventional local plant resources in the ration of animals (Ouedraogo et al., 2021). The use of local feeds rich in proteins and having no competition with humans such as Pueraria phaseoloides, appears as an alternative to commercial classical feeds (Aboh et al., 2002). In fact, Pueraria phaseoloides is a forage rich in fibers and a source of proteins available in tropical zone namely in Gabon. Its protein content varies between 18 and 21% DM and its crude cellulose level varies between 34 and 43% DM (Djago et al., 2007). This forage was subject to many studies on rabbit feeding in Africa (Akoutey et al., 2012 ; Kimsé et al., 2013). All these works showed no negative effect on animals. The objective of this research work was to study carcass characteristics and chemical composition of rabbit meat induced by feeds containing Pueraria phaseoloides.

MATERIALS AND METHODS Experimentation site

The study was conducted from August to December 2018 at the National Higher Institute of Agronomy and Biotechnology (INSAB) of the University of Science and Techniques Masuku (USTM) in Franceville (Haut-Ogooué Province – Gabon). The climate is of equatorial type with two distinct seasons (dry and rainy). The mean annual temperature is 24.6 °C and the humidity level is 80%. This zone is situated at a latitude of 1°37'59'' south, a longitude of 13°35'00'' and an altitude of 405 m above the sea level. The mean rainfall is 1803 mm/year (FDNS, 2004).

Animal resources and housing

In this study, 20 post-weaned female rabbits of local breed aged about 6 weeks with a mean live weight of 611 ± 33.20 g were used. Animals were housed in individual metabolic cages with wire mesh, each measuring 50 cm x 50 cm x 30 cm. Each cage was equipped with a feeder and a drinker.

Plant resources and conduct of trials

Experimental feeds were made up of P. phaseoloides and other feed ingredients. The leguminous plant was harvested at the flowering stage, dried, crushed and then incorporated in different rations. Animals were distributed in 4 treatments (rations) of 5 animals (repetitions) per treatment. The 4 experimental rations (Table 1) were formulated by incorporating gradual levels of flour from P. phaseoloides. The ration R0 contained no P. phaseoloides while the rations R15, R20 and R25 contained 15%, 20% and 25% of flour from P. phaseoloides respectively. Each ration was randomly attributed to 5 animals in a factorial design during 7 weeks. Water was given ad libitum.

Samples of 100 g of each experimental ration was taken and dried at 60 °C till constant weight in a ventilated oven then crushed for chemical analyses. Weighing was done with the help of a digital balance of TEFAL brand mark (capacity 5 kg and precision 1 g). At the end of the study, after a fasting period of 12 hours, the 5 animals of each treatment aged 13

weeks, were weighed to determine mean values of live weights at slaughter. The animals were slaughtered and eviscerated for the evaluation of carcass characteristics.

The determination of the meat yield from the thigh was done by the dissection of the thigh in order to separate bone, muscle and adipose tissues. After obtaining the half carcass, part of the muscles (the majority from the thigh muscles of the rear part, the *Longissimus* muscle, the abdominal muscles of the saddle, the thoracic muscles and the front part of the scapular region) was taken according to experimental rations, then crushed and mixed with a meat crusher. 150 g of the mixture was taken for chemical analyses.

The chemical composition of *P. phaseoloides* and that of rations given to animals were determined in the Laboratory of Research in Biochemistry of the Faculty of Science at the USTM. Analysed parameters were: dry matter (DM) content (%), crude proteins (CB) level (%), crude cellulose (CC)

level (%) and fats content (%). Three repetitions per sample were necessary to determine the mean value of each of these components. As far as rabbit meat is concerned, water, CP, fats and ash contents were equally determined in the same laboratory. DM content was determined using the AOAC method (1990), the CP level using the Bradford/Sedmak method, the fats content using the extraction method with the help of the Soxhlet design and the CC using the Sheerer method.

Statistical analysis

Data on live weights at slaughter, carcass characteristics, weights and yields in organs, meat constitution and bone from the thigh and chemical composition of meat from rabbit were submitted to the analysis of variance at 1 factor (ration) using the SPSS 20.0 software. In case of significant differences between treatments, separation of means was done by the Duncan test at 5% significant level.

Table 1: Formulation and chemical composition of experimental rations and Pueraria phaseoloides.

L	Experimental rations				
Ingredients (%)	RO	R15	R20	R25	
Corn	44.9	44.9	44.9	44.9	
Brewery wet grains	12	6	5	2	
CMAV*	0.1	0.1	0.1	0.1	
Soy flour (44)	2	2	2	2	
Pueraria phaseoloides leaves flour	0	15	20	25	
Cotton-seed cake	9	7	5	4	
Molass	4	4	4	4	
Wheat bran	23	16	14	13	
Fish meal	5	5	5	5	
Total	100	100	100	100	
Chemical composition					Pueraria phaseoloides
Dry matter (% FM)	89.5	90.5	90.7	91.7	88.4
Crude proteins (% DM)	16.7	16.3	16.0	15.9	28.8
Fats (% DM)	2.44	0.71	0.98	2.5	1.54
Crude cellulose (% DM)	13.8	14.0	14.7	15.8	34.6
Ash (% DM)	5.11	4.06	5.20	4.56	5.13
Digestible energy (kcal/kg DM)	2500	2466	2455	2392	449
DE/CP	150	151	153	151	15.6

*CMAV**: Complement of minerals, nitrogen (amino acids) and vitamins, DE: Digestible energy; CP: Crude proteins; FM: Fresh matter; DM: Dry matter.

RESULTS

Carcass characteristics

Carcass characteristics of animals subjected to rations R0, R15, R20 and R25 showed no significant difference (p > 0.05)(Table 2). However, the highest hot carcass yield (81.36%) was recorded with animals receiving ration R0, followed by that of animals fed the ration R20 (80.77%). The highest reference carcass yield (48.84%) was recorded with animals receiving the ration R15, followed by that obtained with animals fed the ration R20 (47.35%). It also appears that the highest yields of the front part and intermediate part (saddle) of the carcass, respectively 18.75% and 9.77%, were recorded in animals fed the ration R15, followed by those animals subjected to the ration R20, respectively 18.64% and 9.36%. Concerning the yield of the pelvic part of the carcass, the highest value (19.19%) was recorded with animals fed the ration R20, followed by that of animals fed the ration R15 (19.02%).

Body organs and organs involved in digestion

With the exception of the yields in sleeves, peri-renal fats, scapular fats, kidneys and skinless head of animals subjected to rations R0, R15, R20 and R25 which showed no significant difference (p > 0.05), the skin and liver yields showed significant differences (p < 0.05) between rations (Table 3). In fact, the skin yield in animals fed rations R15 and R20, respectively 12.89% and 13.30%, were comparable (p > 0.05) between them, but both comparable (p > 0.05) on one hand, to that of animals subjected to the ration R0 (11.82%) and on the other hand, comparable (p > 0.05)to that of animals fed the ration R25 (13.96%). However, the skin yield in animals fed the ration R25 was statistically higher (p < 0.05) than that recorded in animals fed the ration R0. Otherwise, the liver yield in animals fed rations R20 and R25, respectively 2.88% and 2.85%,

were comparable (p > 0.05) between them, but both were on one hand, comparable (p > 0.05) to that of the animals subjected to the ration R0 (3.22%) and on the other hand, comparable (p > 0.05) to that of animals fed the ration R15 (2.59%). However, the liver yield in animals fed the ration R0 was statistically higher (p < 0.05) than that recorded in animals fed with the ration R15.

Thigh constitution

The weight of the thigh, the weight of the thigh meat, the weight of thigh bone and the muscle/bone ratio in animals subjected to rations R0, R15, R20 and R25 showed no significant difference (p > 0.05) (Table 4). However, the highest weight of the thigh (144.00 g) was obtained in rabbits subjected to the ration R20, this weight was followed by that of animals fed the ration R15 (134.33 g). Otherwise, the highest muscle/bone ratio (5.67) was recorded in animals fed rations R15 and R20.

Chemical composition of the meat

Water, fats, proteins and ash contents in meat from animals subjected to the rations R0, R15, R20 and R25 varied according to the incorporation level of P. phaseolides leaves (Table 5). The highest water content (75.05%) FM) was obtained with animals fed the ration R25, followed by that of the animals fed the ration R20 (74.40% FM). On the other hand, lowest fats content (5.41% DM) was recorded in animals receiving the ration R25, followed by that of animals fed the ration R20 (6.67% DM). As far as ash content is concerned, the highest level was obtained in animals receiving the ration R20 (4.17% DM), followed by that of animals fed the ration R25 (4.04% DM). The highest proteins level (23.90% DM) was recorded in animals fed the ration R15, followed by that recorded in those receiving the ration R20 (22.46% DM).

Characteristics		SEM	Prob.			
Characteristics	R0	R15	R20	R25		
Live weight at slaughter (g)	1451.20 ^a	1493.80 ^a	1506.00 ^a	1462.00 ^a	86.20	0.996
Hot carcass (g)	1174.20 ^a	1192.20 ^a	1217.80^{a}	1163.00 ^a	66.57	0.994
Hot carcass yield (with head) (%)	81.36 ^a	79.81 ^a	80.77 ^a	79.75ª	0.33	0.255
Cold carcass (g)	1154.00 ^a	1174.00^{a}	1203.40 ^a	1153.20 ^a	66.57	0.994
Reference carcass (g)	651.00 ^a	703.20 ^a	716.40 ^a	662.00 ^a	46.43	0.959
Reference carcass yield (%)	43.79 ^a	48.84 ^a	47.35 ^a	44.82 ^a	1.82	0.782
Front part of the carcass (g)	243.00 ^a	269.60ª	280.80 ^a	261.20ª	16.98	0.900
Front part yield (%)	16.47 ^a	18.75 ^a	18.64 ^a	17.75 ^a	0.72	0.693
Intermediate part (saddle) (g)	139.00 ^a	139.60 ^a	142.40 ^a	138.00 ^a	11.92	0.999
Intermediate part (saddle) yield (%)	9.16ª	9.77 ^a	9.36ª	9.26 ^a	0.52	0.982
Pelvic part (Rear of the carcass) (g)	267.60 ^a	272.20ª	290.60ª	261.80ª	19.45	0.966
Pelvic part yield (%)	18.04 ^a	19.02ª	19.19 ^a	17.72 ^a	0.80	0.910

Table 2: Characteristics of carcass from local rabbit according to rations.

a: means bearing the same letters on the same line are not significantly different at 5% level; R0, R15, R20 and R25: 0%, 15%, 20% and 25% of incorporation levels of *Pueraria phaseoloides* in rations respectively; SEM: Standard Error on the Mean; Prob.: Probability (5%).

Table 3: Weight and yield of some body organs and those involved in the digestion in local rabbit according to rations.

		Ratio	SEM	Prob.		
Characteristics -	RO	R15	R20	R25		
Skin (g)	177.00 ^a	192.60 ^a	200.20 ^a	208.20 ^a	13.80	0.893
Skin yield (%)	11.82 ^b	12.89 ^{ab}	13.30 ^{ab}	13.96 ^a	0.28	0.037
Sleeves (g)	45.80^{a}	48.00^{a}	46.80 ^a	45.60 ^a	1.93	0.976
Sleeves yield (%)	3.26 ^a	3.25 ^a	3.16 ^a	3.19 ^a	0.07	0.960
Peri-renal fats (g)	14.60 ^a	14.00 ^a	11.40 ^a	12.80 ^a	2.29	0.969
Peri-renal fats yield (%)	1.03 ^a	1.09 ^a	0.91 ^a	1.05 ^a	0.13	0.974
Scapular fats (g)	4.00 ^a	3.60 ^a	4.40^{a}	3.40 ^a	0.71	0.968
Scapular fats yield (%)	0.29 ^a	0.30 ^a	0.32 ^a	0.27 ^a	0.04	0.979
Kidneys (g)	8.20 ^a	8.00^{a}	8.60 ^a	8.40^{a}	0.33	0.941
Kidneys yield (%)	0.57 ^a	0.54 ^a	0.58 ^a	0.59 ^a	0.01	0.652
Liver (g)	45.20 ^a	37.40 ^a	43.40 ^a	42.00 ^a	2.41	0.730
Liver yield (%)	3.22 ^a	2.59 ^b	2.88 ^{ab}	2.85 ^{ab}	0.09	0.004
Skinless head (g)	91.00 ^a	95.00ª	101.60 ^a	88.40 ^a	4.04	0.712
Skinless head yield (%)	6.47 ^a	6.45 ^a	6.81ª	6.20 ^a	0.14	0.524

a et b : means bearing the same letters on the same line are not significantly different at 5 % level ; R0, R15, R20 and R25 : 0%, 15%, 20% and 25% of incorporation levels of *Pueraria phaseoloides* in rations respectively ; SEM : Standard Error on the Mean ; Prob. : Probability (5%).

Characteristics	Rations				SEM	Prob.
	R0	R15	R20	R25		
Thigh weight (g)	130.33 ^a	134.33ª	144.00 ^a	126.00 ^a	12.65	0.975
Meat weight from the thigh (g)	110.00 ^a	114.00 ^a	122.67 ^a	102.33 ^a	11.78	0.959
Bone weight from the thigh (g)	20.33ª	20.33ª	21.33ª	23.67 ^a	1.17	0.774
Muscle/bone ratio	5.33ª	5.67 ^a	5.67 ^a	4.33 ^a	0.45	0.743

Table 4: Meat and bone constitution of the thigh in local rabbit according to rations.

a: means bearing the same letters on the same line are not significantly different at 5% level; R0, R15, R20 and R25: 0%, 15%, 20% and 25% of incorporation levels of *Pueraria phaseoloides* in rations respectively; SEM: Standard Error on the Mean; Prob.: Probability (5%).

Table 5: Chemical composition of meat from local rabbit according to rations.

Characteristics -	Rations							
	R0	R15	R20	R25				
Water content (%FM)	73.51	72.70	74.40	75.05				
Fats content (% DM)	12.34	11.17	6.67	5.41				
Proteins content (% DM)	20.96	23.90	22.46	20.36				
Total ash content (% DM)	3.82	3.61	4.17	4.04				

R0, R15, R20 and R25: 0%, 15%, 20% and 25% of incorporation levels of *Pueraria phaseolides* in the rations respectively; MF: fresh matter; DM: dry matter.

DISCUSSION

Carcass characteristics, body organs and digestive organs

Carcass characteristics of animals subjected to rations R0, R15, R20 and R25 showed no significant difference (p > 0.05). However, the highest slaughter live weight (1506 g) was obtained with rabbits fed the ration R20. This value is higher than that of 1422.12 g reported by Nawel (2007), but lower than that recorded by Lebas (2015) (2376 g). This difference could be explained by the chemical composition of the ration, by the constitution of the diet and by the slaughter age of the animals. In fact, according to Zougou's investigations (2017), the protein level in the ration influences the slaughter weight and the carcass weight. The same observations were reported by Zougou (2012) who observed a variation in live weight at slaughter and in carcass weight with head, by including fresh Tithonia diversifolia leaves in animals' rations. On the other hand, Nawel's works (2007) reported a low live weight at slaughter

(1422.12 g) in rabbits aged 84 days versus a high weight (2125.0 g) in those aged 224 days.

In the present study, the highest hot carcass yield (81.36%) was obtained with animals subjected to the ration R0, followed by that recorded in rabbits fed the ration R20 (80.77%). These results are higher than that reported by Nawel (2007) (67.15%). On the other hand, the highest reference carcass yield (48.84%) was obtained with animals subjected to the ration R15 followed by that recorded in animals fed the ration R20 (47.35%); these values are lower than those reported by Nawel (2007), Abida Ouyed (2009) and Lebas (2015), respectively 53.81%, 51.32% and 56.60%. The highest yields of the front and intermediate carcass, respectively 18.75% and 9.77%, were obtained in the present study with animals fed the ration R15. These values were followed by those obtained in rabbits fed the ration R20, respectively 18.64% and 9.36%. These results are lower than those reported by Lebas (2015) on one hand (31.69% and 17.13% respectively) and, on the other hand, than those recorded by Abida Ouyed (2009) (29.88% and 28.44%

respectively). Moreover, in this study, the highest yield in the pelvic part (19.19%) was obtained in animals fed the ration R20. This value is lower than those reported by Lebas (2015) and Abida Ouyed (2009), respectively 31.15% and 35.74%.

The highest muscle/bone ratio (5.67) was recorded in animals fed rations R15 and R20. This result is comparable to that of 5.87 reported by Lebas (2015), but higher than those recorded by Nawel (2007) and Abida Ouyed (2009) (respectively 4.38 and 4.83). In the present study, the lowest yield in peri-renal fats (0.91%) was recorded with animals subjected to the ration R20 and that of the lowest scapular fats (0.27%) in animals fed the ration R25. These results are lower than those reported by Nawel (2007) who obtained 1.70% of peri-renal fats and 0.74% of scapular fats. These differences could be explained by the type of feed, the slaughter weight, the age of the animals and the breed. In fact, research works carried out by Selmi et al., (2010) revealed that the addition of the yeast Saccharomyces cereviciae in the ration could improve on the carcass yield in lambs. Thus, it has been noticed that carcass weight recorded in supplemented animals (8.46 kg) was higher than that of animals without supplementation (7.97 kg). The increase in feeding during the finishing phase leads to an increase in slaughter live weight, in carcass weight and in carcass yield (Miégoué, 2016). On the other hand, research works conducted by Zougou (2012) revealed that the slaughter live weight is significantly (p > 0.05) correlated to carcass yields and that, those parameters are influenced by feeding. Investigations from Heyer and Lebret (2006) showed that the increase in the age at slaughter goes hand in hand with the improvement in carcass yield. Concerning the breed, Liviu (2005) noticed that, carcass weight was higher in hybrid lambs (15.7 kg) than in homozygotes (13.2 kg).

Chemical composition of meat

The meat with highest water content in this study was obtained in animals subjected to the ration R25 (75.05%). This result is higher than those reported by Nawel (2007) and Combes (2004), respectively 72.25% and 73.5%; but close to that obtained by Tandzong et al. (2015) (74.31%) in cavies fed a ration containing 8% of cassava leaves. However, it was lower than that reported by Zougou (2017) and Tandzong et al. (2015), respectively 92.00% in cavies fed a ration containing 14% DM of CP and 77.79% in cavies fed a ration containing 10% of cassava leaves. This difference could be justified by the la chemical composition of rations, the animal species, the breed, the sex and the type of muscle. In fact, the chemical composition of meat varies according to rations (Tandzong et al., 2015; Mweugang, 2016) and according to the level of CP in the ration (Zougou, 2017). On the other hand, research works from Tandzong et al., (2015) revealed that the water content in male muscles was higher (70.45 - 73.23%) than in females (65.56 - 73.23%). Moreover, research works from Tandzong et al. (2015) highlighted that the water content in thigh muscles was higher (72.31%) than in the loin (70.89%) and the shoulder (69.77%). The same in observation was made by Zougou (2017) who noticed that the water content in thigh muscles was higher (90.00%) than that of the loin (89.83%) and that of the shoulder (83.33%). Investigations from Combes (2004) showed a water content of 69.1% in calf meat and 72.2% in chicken.

The lowest fat content in meat in this study (5.41% DM) was obtained in animals fed the ration R25, followed by that of animals fed the ration R20 (6.67% DM). These values are close to those obtained by Combes (2004) (5%), but higher than those reported by Nawel (2007) (3.47%). This difference could be explained by the age at slaughter, the sex and the meat slice. In fact, the mean fats content in rabbit meat is 5 g/100g, however according to the metabolic type, the anatomical location or the function of the muscles, fats content in muscles varies from 0.9 to 5 g/100 g (Nawel, 2007). Fats content in muscles increases with age and the weight of animals (Heyer and Lebret, 2006). According to the same authors, when the body weight increases from birth to slaughter, variations in carcass composition are

observed. Moreover, the increase in slaughter weight leads to a decrease in muscles and bones proportions, and increase in the proportion of fats in the carcass. Research findings from Tandzong et al. (2015) and Zougou (2017), revealed that in a same animal, fats content can vary according to the muscle type. The sex of the animal induces different body compositions (Zougou, 2017). According to this author, females have a lower growth potential than that of entire males, but the development of adipose tissues is more rapid. Thus, with the same live weight, females have a high fat deposition in adipose tissues than males (Tandzong et al., 2015; Zougou, 2017). The highest fats content in female muscles could be explained by hormone effects such as oestrogens (Hottin et al., 2019). In fact, oestrogens stimulate fats distribution in the pelvic cavity and in thigh.

Highest proteins contents in this study were recorded in animals fed the ration R15 (23.90%). This value is higher than that reported by Combes (2004) and Nawel (2007) (22.19%). The crude proteins in the ration, the age and the muscle type could justify this difference. In fact, investigations from Zougou (2017) revealed that the chemical composition of cavy meat varied according to the level of proteins in the ration. This author reported proteins levels of 18.50%, 17.00% and 19.00% in the meat of animals receiving rations containing respectively 14%, 16% and 18% of CP. On the other hand, Nawel's investigations (2007) revealed proteins levels of 18.2%, 20.2% and 22.19% in rabbits aged respectively 30, 70 and 84 days. Research works of Tandzong et al. (2015) showed that, proteins level in thigh was higher (18.39% DM) than that of the loin (18.35% DM) and of the shoulder (17.34% DM); the same observation was made by Zougou (2017).

The highest ash content in meat in this study (4.17%) was obtained with animals fed the ration R20. This value is higher than those reported by Nawel (2007) and Combes (2004), respectively 1.28% and 1.2%. This difference

could be related to the feeding and animal species.

Conclusion

At the end of this study, it emerges globally that, the incorporation of *Pueraria phaseoloides* in rabbit feeding had no influence on carcass characteristics. On the other hand, the chemical composition of meat varied according to the incorporation level of *P. phaseoloides* leaves. Based on these results obtained, the ration containing 20% of *P. phaseoloides* (R20) could be recommended. However, it would be desirable to continue this study over a long period in order to better assess carcass characteristics and the chemical composition of rabbit meat.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

GCZT designed, the set up experimental design, participated in the conduct of trials and wrote the manuscript ; AIT participated in setting up the experimental design and in the conduct of trials as well as in the critical reading of the manuscript ; FNM, AVM participated in the revision of the manuscript and the scientific assistance in the realization of this work; MT set up the experimental design, conducted the trials, conducted all laboratory analyzes under the guidance of LCOE and collected the data; MK, CSO, LCOE, J-PO, FCNN, FT, EM participated in the technical and scientific assistance of the work as well as in the critical reading of the manuscript; BB and EPT coordinated and guided the technical and scientific realization of the work.

All the authors have read and approved this version of the work.

ACKNOWLEDGEMENTS

We sincerely thank the National Higher Institute of Agronomy and Biotechnology (INSAB), University of Science and Techniques Masuku (USTM) to have financed this study.

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