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Indicators of kidney function in guinea pigs (*Cavia porcellus*) fed with the inclusion of pisonay (*Erythrina edulis*) meal of three regrowth age

Indicadores de la función renal en cuyes (*Cavia porcellus*) alimentados con la inclusión de harina de pisonay (*Erythrina edulis*) de tres edades de rebrote

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Article Data

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Abstract

The objective of the study was to determine the serum levels of creatinine, blood urea nitrogen (BUN) and the kidney weight ratio of guinea pigs (*Cavia porcellus*) fed with the inclusion of pisonay (*Erythrina edulis*) meal of three regrowth ages in the Mosoccpampa sector, Apurímac. Eighty improved male guinea pigs we´re used, which we´re randomly distributed into groups of 8 guinea pigs for each dietary treatment containing 10, 20 and 30 % inclusion of pisonay meal for each regrowth age of 4, 8 and 12 months and a control group with 20 % alfalfa meal under isoproteic and isoenergetic conditions. Blood samples we´re taken to determine serum activity using commercial kits (Valtek Diagnostics) for photometry. The data we´re analyzed under the completely randomized design and for the comparison of means, the Dunnett´s test was applied ($p \leq 0.05$). Serum creatinine levels of the D_2, D_3, D_4 and D_8 diets (0.61, 0.68, 061 and 0.66 mg dL^{-1} respectively) were different from the control group (0.48 mg dL^{-1}) ($p<0.05$). BUN levels of the D_1 (21.26 mg dL^{-1}) and D_7 (14.05 mg dL^{-1}) diets were different from the control group (17.56 mg dL^{-1}) ($p<0.05$). The kidney weight ratio in the diets (0.85 %) was similar to control diet (0.87 %) ($p>0.05$). The values of serum creatinine, BUN and kidney weight ratio we´re not affected by the inclusion of pisonay meal of three regrowth ages in the diet, this behavior would indicate that pisonay meal could be considered as input to produce integral food for guinea pigs in the growth stage.

Resumen

El objetivo del estudio fue determinar los niveles séricos de creatinina, nitrógeno ureico en sangre (NUS) y la relación riñón peso vivo de cuyes (*Cavia porcellus*) alimentados con la inclusión de harina de pisonay (*Erythrina edulis*) de 3 edades de rebrote en el sector de Mosoccpampa, Apurímac. Se utilizaron 80 cuyes machos mejorados, que fueron distribuidos al azar en grupos de 8 cuyes para cada tratamiento dietético que contenía 10, 20 y 30 % de inclusión de harina de pisonay (HP) por cada edad de rebrote de 4, 8 y 12 meses y un grupo control con 20 % de harina de alfalfa en condiciones isoprotéicas e isoenergéticas. Se tomaron muestras de sangre con finalidad de determinar la actividad sérica mediante kits comerciales (Valtek Diagnostics) para fotometría. Los datos fueron analizados bajo el diseño completamente al azar y para la comparación de medias se aplicó el contraste de Dunnett's ($p \leq 0.05$). Los niveles séricos de creatinina de las dietas D_2, D_3, D_4 y D_8 (0.61, 0.68, 061 y 0.66 mg dL^{-1} respectivamente) fueron diferentes al grupo control 0.48 mg dL^{-1} ($p<0.05$). Los niveles de NUS de las dietas D_1 21.26 mg dL^{-1} y D_7 14.05 mg dL^{-1} fueron diferentes al grupo control 17.56 mg dL^{-1} ($p<0.05$). La relación riñón peso vivo en las dietas (0.85 %) fueron similares a la dieta control 0.87 % ($p>0.05$). Los valores de creatinina sérica, NUS y la relación riñón peso vivo no fueron afectados por la inclusión de HP de 3 edades de rebrote en la dieta, esté comportamiento nos indicaría que la HP podría ser considerado como insumo para elaborar alimento integral para cuyes en la etapa de crecimiento

Introduction

Guinea pig meat is an alternative for consumption with respect to other types of meat. It's being successfully raised on the coast and high Andean areas of Peru, they are a source of income for producers, and the feeding of guinea pigs can vary, from the use of whole food on the coast, and in the highlands the use of alfalfa or other fodder, or a combination of both[1]. The national guinea pig population in 2012 was estimated at 1 012 181 and a per capita consumption of 0.35 kg/inhabitant/year[2], in 2017 the guinea pig population in Peru was 17 380 000 animals[3] and is currently mentioned above 20 million, the Apurímac region would rank fourth in number of guinea pigs.

The genus *Erythrina* contains in its leaves alkaloids, flavonoids, terpenoids[4], tannins and total polyphenols[5], antinutritional factors, which can cause cytotoxic effects[6], and could affect the weight of internal organs. The foliage of trees, shrubs and bushes in hay and ground has shown its nutritional and productive qualities as an input in the production of concentrated feed for guinea pigs[7,8]. Such as the leaves of *E. edulis* (pisonay), with a nutritional composition of the fresh forage (wet basis), with respect to dry matter (DM) 25.39 %, crude protein (CP) 7.65 %, crude fiber 7.35 %, crude fat 0.31 % and ash 1.29 %[9]. Also, in leaves and petioles of *Erythrina* sp., pruned from trees used for animal feed, DM ranged from 24.8 to 31.7 %, CP from 20.1 to 23.5 %, crude fiber 7.35 %, crude fat 0.31 % and ash 1.29 %. Ethereal extract from 0.4 to 2.5 %, ash from 8.6 to 11.6 % neutral detergent fiber was most stable at 58.0 % and acid detergent fiber from 32.6 to 34.7 %[10,11]. The inclusion of forages and non-conventional feedstuffs such as meal in the guinea pig diet did not modify the productive behavior or the quality of the feed[12]. *Erythrina* leaves as meal in guinea pig feed caused variations in the profiles of total protein, blood albumin and carcass yield[13]. Domestic rodents with signs of renal insufficiency, high levels of BUN and creatinine serum were observed[14]. In addition, in guinea pigs with renal functional impairment was manifested by a doubling of creatinine concentration serum and increased of BUN[15]. In degenerative and metabolic diseases affecting the urinary system are so far analyzed in the context of laboratory rodents[16].

Biochemical studies and the analysis of organ weight through the organ-body weight ratio are important to evaluate the toxicity of antinutritional factors, in rabbits fed with the inclusion of *Gliricidia sepium* leaf meal in proportions of 10 and 15 % did not cause variations in the values of internal organs, expressed as a percentage of live weight[17]. *Mucuna utilis* in graded levels did not present a significant difference (P>0.05) in the weight of organs[18]. 20 % of *Moringa oleifera* in the dietary intake of caused a downward trend in live weight and kidney weight[19]. In this sense, due to the nutritional benefits, the use in animal feed and possible toxicity of pisonay, our objective was to determine the serum levels of creatinine, BUN and the live weight kidney ratio of guinea pigs (*C. porcellus*) fed with the inclusion of pisonay meal at three different ages of regrowth.

Materials and methods

The work was carried out in a guinea pig shed, built with adobe material and an area of 200 m^2, located in Mosoccpampa, Tamburco, at an altitude of 2800 masl, the minimum and maximum temperatures were 6.8

and 23.7° C, the annual rainfall was 1022 mm and the relative humidity was 73.6 %. In addition, biosecurity protocols we´re applied to prevent diseases in the guinea pigs[20].

The guinea pigs were from the La Inmaculada farm (commercial breeding) located in Curahuasi, which it registered in the Regional Directorate of Agriculture of Apurimac, 80 type I male guinea pigs of approximately 15 days of age we´re used, with an average live weight of 324.05±37.23 g, which we´re randomly distributed to 10 experimental groups.

The guinea pigs we´re raised in one-story cages with an area of 0.81 m^2, the living space for each guinea pig was (0.20 m^2)[21], the cages we´re provided with hopper type feeders with a metal base and corduroy type drinkers.

The leaves and petioles we´re harvested by pruning pisonay (*E. edulis*) trees located from area; the characteristics observed in the trees we´re described by Cárdenas-Villanueva[22]. Normally used for animal feed as fresh forage, pruning was carried out according to the last cut for each age of regrowth (4, 8 and 12 months), the foliage was subjected to shading for approximately 30 days, after which it was milled in a hammer mill with a 2 mm sieve.

Nine balanced diets were prepared under isoproteic and isoenergetic conditions (Table 1), 3 for each age of pisonay regrowth and 1 control diet with the inclusion of alfalfa meal (D_0). The diets were prepared using wheat bran, alfalfa meal (20 %) and pisonay meal (10, 20 and 30 %), ground corn, soybean cake, and the following additives: dicalcium phosphate, calcium carbonate, common salt, vitamin C, myco- sequestering agent, premix (vitamins and minerals) and DL-Methionine, which we supplied by Del Corral enterprise.

Table 1 Calculated nutritional composition of diets for guinea pigs

Nutrients	D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9
Dry matter, %	93.4	93.7	93.6	93.5	93.6	93.6	93.5	93.6	93.6	93.8
Crude protein, % DM	17.4	17.9	17.7	17.5	17.8	17.7	17.6	17.9	17.8	17.8
Ethereal extract, % DM	1.8	2.3	2.0	1.9	2.3	2.1	1.9	2.4	2.3	2.3
Ash, % DM	4.3	4.6	4.8	5.1	4.6	4.9	5.4	4.6	5.2	5.6
Neutral detergent fiber, % DM	32.3	36.0	33.6	31.7	36.0	33.9	32.0	36.1	34.2	32.6
Digestible energy, Mcal/kg	3.06	2.96	3.01	3.01	2.97	3.01	3.01	2.98	2.98	3.01

The diets we fed in meal, 7 days in the habituation phase and 56 days in the experimental phase, in each phase the clinical status of the animals was evaluated, the guinea pigs were fed without restriction once a day and fresh water was offered ad libitum.

After the conclusion of the experimental phase (56 days) all the guinea pigs we benefited, the blood of each guinea pig was collected directly from the jugular vein in test tubes without anticoagulant.

Previously the guinea pigs were insensitized[23], then the blood of each guinea pig was centrifuged (Hettich Rotofix 32A) to obtain blood serum, which was transferred to 5 mL vials and frozen at -20° C (Boch no frost freezer), and then creatinine and BUN were determined by means of a semi-automated biochemical analyzer (Stat Fax 3300).

To determine the serum levels of creatinine and BUN, 2 repetitions were performed for each blood serum sample from all guinea pigs, using the procedures proposed by the commercial firm Valtek Diagnostics, Chile, containing working reagent and standard or calibrator.

For the kidney live weight ratio, the guinea pigs were weighed at the end of the experiment on a Henkel BRD04kF digital balance (± 1.0 g). After the benefit, the kidneys of each of the guinea pigs were extracted and weighed on an Ohaus Ad- venturer AX5202 analytical balance (± 0.01 g), and the ratio was determined according to the following formula:

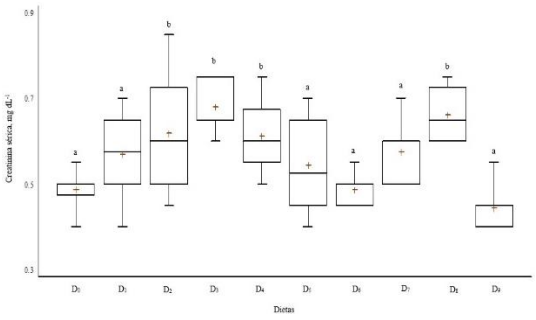
$$\text{Rel}\frac{\text{R}}{\text{LW}}(\%) = \frac{\text{Weight of kidneys}}{\text{Final live weight}} \text{x } 100$$

To perform the analysis of variance, these percentage values, being below 30 %, were subjected to the angular or Bliss transformation.

The normality of the serum levels and the live weight-ratio of each of the diets was evaluated by the d'Agostino-Pearson test, the data were analyzed under a completely randomized design and for the comparison of means the Dunnett's test was applied ($p \leq 0.05$), previously the test of homogeneity of variances was performed through Levene's test. In addition, the linear correlation between the 3 variables was performed.

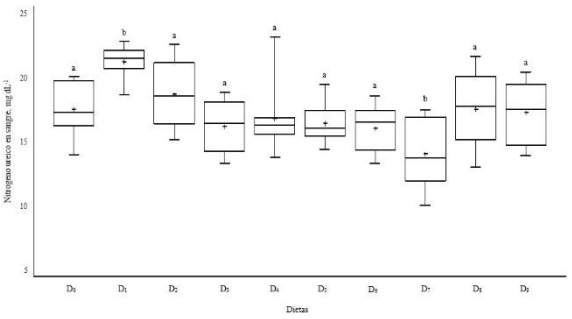
Results

Figure 1 Serum creatinine levels in guinea pigs according to diet



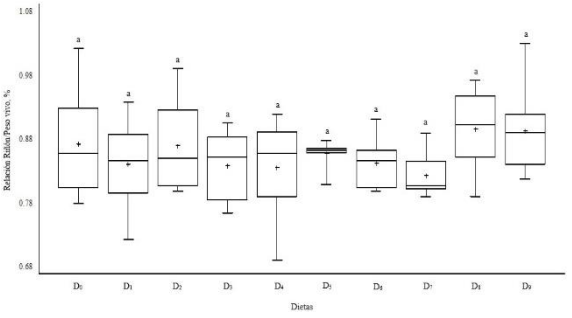
The serum creatinine levels (Figure 1) of the diets D_2 (0.61±0.14 mg dL^{-1}), D_3 (0.68±0.05 mg dL^{-1}), D_4 (0.61±0.08 mg dL^{-1}) and D_8 (0.66±0.20 mg dL^{-1}) were different to D_0 (0.48±0.04 mg dL^{-1}) ($p<0.05$). In diet D_2 the highest extreme value of 0.85 mg dL^{-1} was observed in several diets the lowest value was 0.40 mg dL^{-1}.

Figure 2 Blood urea nitrogen in guinea pigs according to diet



The BUN levels (Figure 2) of the diets D_1 (21.26±1.33 mg dL^{-1}) and D_7 (14.05±2.78 mg dL^{-1}) were different from the control diet (17.56±2.15 mg dL^{-1}) ($p<0.05$). The most extreme value was observed in diet D_4 (23.15 mg dL^{-1}) and the lowest value was in D_7 (10.00 mg dL^{-1}).

Figure 3 Kidney to live weight ratio of guinea pigs by diet



The effect of the diets with the inclusion of pisonay meal was not significant ($p>0.05$) in the live weight kidney ratio (Figure 3) of guinea pigs with respect to the D_0 diet (0.87 %). The most extreme value was observed in diet D_9 (1.02 %) and the lowest value was in D_4 (0.74 %). The correlation between the variables creatinine and live weight kidney ratio (Table 2) was significant ($p<0.05$), which suggests that there is a

negative linear relationship and the strength of the linear correlation was (r: ±0.09 to ±0.00) between BUN and kidney live weight ratio (p>0.05).

Table 2 Linear correlation between study variables

		Creatinine, mg dL^{-1}	BUN, mg dL^{-1}
Creatinine, mg dL^{-1}		.1047 p = .3554	-.2407 p = .0315
BUN, mg dL^{-1}	.1047 p = .3554		-.0387 p = .7335
K/LW Ratio, %	-.2407 p = .0315	-.0387 p = .7335	

BUN: Blood urea nitrogen. K/LW: Kidney live weight ratio. p: probability.

Discussion

With respect to serum creatinine, the values by effect of diets D_2, D_3, D_4 and D_8 would be close to the lower limit of 0.6 mg dL^{-1} proposed by Gross[24]. All diets remained in the ranges (0.1 to 0.9 mg dL^{-1}) in 13/N hybrid male guinea pigs[25]. Diets D_0, D_6 and D_9 remained between 0.33 to 0.51 mg dL^{-1} values in Weiser-Maples male guinea pigs[26] and the serum creatinine levels due to the effect of the diets would not be in the range (0.75 to 2.55 mg dL^{-1}) referential for albino guinea pigs[27], these variations would probably be due to the species under study and age.

This amplitude in the ranges for creatinine suggests that the inclusion of pisonay meal from 3 ages of regrowth would not cause significant changes or toxicological implications. As occurred with pisonay supplied to guinea pigs as fresh fodder above 50 % which increased serum creatinine levels above (3.1 mg dL^{-1})[28]. Also *Lantana cámara* as a non-conventional fodder, which is consumed in times of scarcity, increased creatinine levels (1.21 mg dL^{-1}) with respect to the control group (0.54 mg dL^{-1})[29]. With vegetable-based diets caused creatinine to reach up to 4.2 mg dL^{-1} probably due to the presence of oxalates[30] and with mixed feeding, fresh alfalfa was included, offered there was no evidence of renal damage in male guinea pigs[31]. This increase in serum creatinine could be related to mild exposure to nephrotoxic substrates that would cause subchronic toxicity[29,32] in a given time to chronic kidney disease where 75 % of renal function is lost[33]. The BUN due to the effect of the diets was similar to the range of 17 to 29 mg dL^{-1} for 13/N hybrid male guinea pigs[25]. With the exception of D_7 and in all cases was in the range (12.5 to 41.8 mg dL^{-1}) referential for albino guinea pigs[27]. Opposite occurs when contracted with the range (26.6 to 37.3 mg dL^{-1}) found in Weiser-Maples male guinea pigs[26].

The reported values of BUN were similar to the values with the inclusion of fresh forage from pisonay in the diet for guinea pigs where it was obtained from (12.8 to 16.8 mg dL^{-1})[28]. Diets based on vegetables in the guinea pig diet, a double increase was observed with respect to the normal value for BUN, probably due to the ingestion of vegetables containing oxalate[30]. These variations in the values found, would be insufficient to assert the presence of renal disease, the BUN would increase from 15 to 40 times in guinea pigs[34] as would occur in renal failure[33]. The inclusion of pisonay meal up to 30 % would not result in nephrotoxicity, this anomaly would be subject to adult guinea pigs, young

immunocompromised or physiologically stressed guinea pigs[35].

The inclusion of pisonay meal did not result in high levels of creatinine serum and BUN. These values are in the normal reference range for guinea pigs, which would indicate that glomerular filtration and secretion in the proximal tubule would be normal and would not cause acute renal damage[36]. On the other hand, we could assume that the extent of renal damage was tolerable in the initial and experimental phase since no mortality was observed in the guinea pigs.

The kidney/live weight ratio remained stable as the percentage of pisonay meal of each regrowth age increased. Being congruent with the observation in apparently healthy adult male guinea pigs, reared in captivity and fed with food pellets, carrots and fruits only, which had a ratio of 0.88%[37]. Similarly, the inclusion of 50 and 75 % of potato plants in the diet of guinea pigs, after 54 days of experimentation, the ratio decreased from 0.90 to 0.80 % respectively ($p<0.05$)[38]. Is contradictory, when compared with male guinea pigs of the Peru line that received ground flaxseed (100 g) in isonitrogenous concentrates plus alfalfa for 30 days, they showed a ratio of 1.02 %[39]. In another study, the weight of gastrointestinal tract, kidneys and lungs (g/100 g of body weight) of male guinea pigs of the Peru breed, after 42 days, had a slight decrease due to the effect of diets with different dietary electrolyte balance, which also moderately increased the levels of creatinine and BUN[40].

This is corroborated by the final live weight observed the guinea pigs achieved 1032.38±111.08 g and with the control diet they reached weights of 1031.50±167.81 g. This behavior can be associated to the adequate protein level of the diets that did not cause an increase in the value of the BUN[41], which remained within the normal range for guinea pigs. Furthermore, the similarity in body weight would be related to the physiological response of adaptation to the possible toxic effects, which did not cause a reduction in food consumption and therefore in the absorption of nutrients[42].

The negative linear correlation observed between creatinine and Rel/PV ratio ($p<0.05$) and the absence of significant difference in Rel/PV ratio, would indicate that the relative weight of vital organs such as kidneys would be in normal conditions[43], therefore, there would be no toxic effects due to the inclusion of HP in the diet of growing guinea pigs.

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Conflicts of interest

The authors have no conflict of interest.

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Ethical considerations

We considered the vital space of the cages that allowed the welfare and tranquility of the guinea pigs, adequate diets were provided in accordance with nutritional requirements and fresh water was freely available, finally the guinea pigs were fed according to current regulations.

Authors' contribution to the article

Jennefer Vega Cruz, experiment execution, drafting and preparation of the original draft. *Ruth Ramos- Zuñiga*, experiment execution and project administration. *Ludwing Angel Cárdenas-Villanueva*, supervision of results and discussion, information search, writing and final revision of the document for possible publication.

Research limitations

There were no limitations to the research.

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