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Original

Hepatic biochemical profile in guinea pigs fed with pisonay (*Erythrina edulis*) meal

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ABSTRACT

Objective. Evaluate the variations of the inclusion of *Erythrina edulis* leaf meal (EELM) of three regrowth ages (Ra) on the hepatic metabolites of guinea pigs. **Materials and methods.** 100 improved male guinea pigs assigned to ten diets-treatments were used: (T0) 20% lucerna meal, (T1) 4 Ra/10% EELM, (T2) 4 Ra/20% EELM, (T3) 4 Ra/30% EELM, (T4) 8 Ra/10% EELM, (T5) 8 Ra/20% EELM, (T6) 8 Ra/30% EELM, (T7) 12 Ra/10% EELM, (T8) 12 Ra/20% EELM, (T9) 12 Ra/30% EELM. At 56 days, blood samples in order to determine total protein, albumin, total bilirubin, aminotransferases, alkaline phosphatase, and gamma-glutamyl transpeptidase they collected and were analyzed undering completely randomized design. In addition, was used the factorial arrangement to evaluate the Regrowth Age Factor and the Inclusion Levels Factor. **Results.** The diets-treatments and the inclusion of EELM did not affect the serum concentration of total protein ($p>0.05$). The diets-treatments, regrowth age and inclusion did not affect alkaline phosphatase ($p>0.05$). The inclusion had no effect on albumin and gamma-glutamyl transpeptidase ($p>0.05$). The concentration of total bilirubin and aminotransferases were affected by the diets-treatments and decreased with increasing age at regrowth and the inclusion of EELM ($p<0.05$). Albumin and gamma-glutamyl transpeptidase increased due to the effect of regrowth age ($p<0.05$). **Conclusions.** The inclusion of EELM from three regrowth ages does not cause variations in hepatic metabolites since they are within acceptable levels for guinea pigs.

Keywords: Alkaline phosphatase; aminotransferases; gamma-glutamyl transpeptidase; leaves; petioles; total bilirubin (*Sources: MeSH, NLM*).

RESUMEN

Objetivo. Evaluar las variaciones de la inclusión de harina de hojas de Erythrina edulis (HHEE) de tres edades de rebrote (Ed) sobre los metabolitos hepáticos de cuyes. **Materiales y métodos.** Se utilizó 100 cuyes asignados a diez dietas-tratamientos: (T0) 20% harina de alfalfa, (T1) 4 Ed/10% HHEE, (T2) 4 Ed/20% HHEE, (T3) 4 Ed/30% HHEE, (T4) 8 Ed/10% HHEE, (T5) 8 Ed/20% HHEE,

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(T6) 8 Ed/30% HHEE, (T7) 12 Ed/10% HHEE, (T8) 12 Ed/20% HHEE, (T9) 12 Ed/30% HHEE. A los 56 días se recolectaron muestras de sangre con finalidad de determinar la proteína total, albumina, bilirrubina total, aminotransferasas, fosfatasa alcalina y gamma-glutamil transpeptidasa y fueron analizados bajo un diseño al azar. Además, se utilizó el arreglo factorial para evaluar el Factor Edad de rebrote y el Factor Niveles de inclusión. **Resultados.** Las dietas-tratamientos y la inclusión de HHEE no afectó la concentración sérica de la proteína total ($p>0.05$). Las dietas-tratamientos, la edad de rebrote y la inclusión no afectó la fosfatasa alcalina ($p>0.05$). La inclusión no tuvo efecto sobre la albumina y gamma-glutamil transpeptidasa ($p>0.05$). La concentración de bilirrubina total y aminotransferasas fueron afectadas por las dietas-tratamientos y disminuyeron al incrementarse la edad de rebrote y la inclusión de HHEE ($p<0.05$). La albumina y gamma-glutamil transpeptidasa se incrementaron por efecto de la edad de rebrote ($p<0.05$). **Conclusiones.** La inclusión de HHEE de tres edades de rebrote no provoca variaciones en los metabolitos hepáticos ya que se encuentran dentro de los niveles aceptables para los cuyes.

Palabras clave: Aminotransferasas; bilirrubina total; fosfatasa alcalina; gamma-glutamil transpeptidasa; hojas; peciolos (*Fuentes: MeSH, NLM*).

INTRODUCTION

Fresh forage from the Genus Erythrina is used as an alternative in feeding guinea pigs (1). Also, its use has been observed in cattle feeding in winter times, where fodder is insufficient, by the breeders of the inter-Andean valley of Abancay, who, due to its nutritional components, use *Erythrina* sp (pisonay), In addition, being as living fences, it has been shown that the leaves and petioles had values of 20.1 to 26.1% crude protein, neutral detergent fiber reached up to 58.6% at twelve months of regrowth age (2), the calculated digestible energy was 2.6 Mcal/kg DM, in addition, with the inclusion of fresh fodder in the guinea pig feed, 8 g/day and 64% carcass yield were achieved (3), another option is the addition of meal from the fodder of the *Erythrina edulis* (pajuro bean) as a dietary supplement for guinea pigs, the inclusion of 2% in the concentrate, managed to optimize the feed conversion in 1.7 and carcass yield in 75.9% (4) likewise, the inclusion of *Erythrina poeppigiana* (Caraca) in the form of flour is adequate to be added to the integral feed for guinea pigs (5).

The consumption of fresh forage of pisonay meal after 21 days, in proportions above 50%, would cause the presence of abnormalities in the liver and increase the enzymes alanine (61.9 IU/L) and aspartate (76.3 IU/L) aminotransferases, due to the presence of tannins and alkaloids in the leaves, would indicate probable toxicity (6), in other studies, it has been observed that the leaves of *Erythrina senegalensis* showed the presence of saponins and flavonoids, in addition to terpenoids, which would not cause acute toxicity in rodents (7), on the other hand, the extract of *Erythrina variegata* in Wistar rats, caused slight changes in total protein, albumin, total bilirubin, and aspartate aminotransferase without moving away from normal ranges, except for the levels of alanine aminotransferase, these variations would indicate that the damage was reversible at the liver level and would not cause subchronic toxicity (8).

The guinea pig is used to test the effects of diseases related to diet (fatty liver), cardiovascular, and others (9), the determination of total protein, albumin, and total bilirubin are used as tests of various liver functions, alanine, and aspatate aminotransferases are markers of hepatocellular damage, in addition, alkaline phosphatase and gamma-glutamyl transpeptidase are markers of cholestasis, these metabolites can help diagnose hepatobiliary problems in their primary or secondary stage (10), these indicators were used in rodents to assess the toxicity of hydroalcoholic substances from the genus Erythrina (11) as well as the effects of various degrees of inclusion of leaf meal and other fractions of unconventional forage plants in the diet, to achieve adequate growth of guinea pigs (12).

Therefore, the objective was to evaluate the variations of the inclusion of *Erythrina edulis* leaf meal (EELM) of three regrowth ages (Ra), as an input in the integral feed for guinea pigs, on the hepatic metabolites.

MATERIALS AND METHODS

Location. The experiment was carried out in a guinea pig shed, with dimensions of 21.0x9.5x3.0 m and oriented from south to north, located in the Moccospampa sector belonging to the province of Abancay, Apurimac, Peru, located at 13°37′ south latitude and 72°52′ west longitude, the climatic conditions that took place at an altitude of 2880 m, with respect to the annual precipitation was 1022 mm, the relative humidity of 73.6% and the temperature of 6.8 to 23.7°C.

Animals and accommodation. 100 whole improved male guinea pigs of 15 days of age, with weights of 324.05±37.23 g, were used, randomly assigned to 10 experimental groups. The guinea pigs were raised in aerial-type cages (0.9x0.9x0.4 m), with a living space of 0.20 m^2 per guinea pig, which provided adequate conditions for the animals (13).

Production of pisonay meal. Leaves and petioles of *Erythrina edulis* (pisonay) trees of three regrowth ages (4, 8, and 12 months) were used, trees located as living fences and pruned from the last cut, which were subjected to drying in the shade for approximately 30 days, subsequently, the dry forage was ground with a hammer mill (Maquitaxi, PE) with a 2 mm sieve.

Experimental diets. Ten experimental diets were prepared (Table 1), considering the nutritional requirements proposed by the NRC (1995). The guinea pigs underwent an adaptation stage for 7 days and 56 days of experimentation. In hopper-type feeders, whole food was offered once a day and bell-type drinkers were used to providing fresh water *ad libitum*.

The treatments included 10, 20, and 30% from *Erythrina edulis* leaves meal (EELM) of three ages (4, 8, and 12 months) of regrowth (Ra) corresponding to (T0) 20% alfalfa meal, (T1) 4 Ra/10% EELM, (T2) 4 Ra/20% EELM, (T3) 4 Ra/30% EELM, (T4) 8 Ra/10% EELM, (T5) 8 Ra/20% EELM, (T6) 8 Ra/30% EELM, (T7) 12 Ra/10% EELM, (T8) 12 Ra/20% EELM, (T9) 12 Ra /30% EELM.

Table 1. Percentage of food inputs and nutrients calculated from the experimental diets.

Supplies	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9
		Regrowth age (months)								
		4			8			12		
		Inclusion (%)								
		10	20	30	10	20	30	10	20	30
Pisonay meal		10.0	20.0	30.0	10.0	20.0	30.0	10.0	20.0	30.0
Alfalfa meal	20.0									
Wheat bran	46.0	58.1	39.9	22.0	58.2	40.3	22.6	59.2	42.2	25.4
Soya cake	18.4	17.3	16.2	14.5	17.3	16.2	14.3	17.4	16.1	14.6
Cornmeal	11.9	11.9	21.3	31.4	11.9	21.0	31.0	10.9	19.2	27.9
Dicalcium phosphate	1.4		1.2	1.0		1.2	1.0		1.2	1.0
Calcium carbonate	0.6	1.6	0.4		1.6	0.4		1.6	0.4	
Common salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin C	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Micro sequestrant	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Minerals and Vitamins	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DL- Methionine	0.1	0.02	0.1	0.17	0.02	0.1	0.17	0.02	0.1	0.17
Calculated Nutrients										
Dry material, %	93.4	93.7	93.6	93.5	93.6	93.6	93.5	93.6	93.6	93.8
Crude protein, % MS	17.4	17.9	17.7	17.5	17.8	17.7	17.6	17.9	17.8	17.8
Digestible energy, Mcal/Kg	3.06	2.96	3.01	3.01	2.97	3.01	3.01	2.98	2.98	3.01

Biochemistry in blood serum. After 56 days, the guinea pigs benefited, prior to desensitization of the animals due to dislocation of the neck vertebrae, (14) and the blood were collected in test tubes without additives, obtaining the serum was by centrifugation (Hettich Rotofix 32A, KG) at 1398 x g for 10 minutes, stored at -20°C and later determining the total protein (TP) and albumin (ALB) in triplicate. Total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP) were performed in duplicate and a single reading for gamma-glutamyl transpeptidase (GGT), reagent protocols according to the manufacturers (Valtek Diagnostics, Chile), using the UV/Vis spectrophotometer (Jeyway 6405, UK) and TB in a semi-automated biochemical analyzer (Stat Fax 3300, USA).

Statistical analysis. Serum levels of hepatic metabolites were analyzed under a completely randomized design with ten treatments, normality was evaluated with the Shapiro-Wilk test, and Dunnett's contrast ($p \leq 0.05$) was applied in the comparison of means, previously the variance homogeneity test was performed through Levene. In addition, the factorial arrangement was used to evaluate the Regrowth Age Factor (4, 8, and 12 months) and the Inclusion Levels Factor (10, 20, and 30%).

RESULTS

Individual tests regarding serum protein (T0: 5.42 vs T: 5.80 g/dL) and alkaline phosphatase (T0: 118.16 vs T: 130.23 IU/L) did not cause variations and were similar ($p>0.05$), except for the albumin (T0: 3.03 vs T: 2.81 g/dL), total bilirubin (T0: 0.17 vs T: 0.27 mg/dL) and aminotransferases (ALT, T0: 35.93 vs T: 57.84 IU/L; AST, T0: 63.94 vs T: 83.56 IU/L) had a variable behavior with respect to the control diet ($p<0.05$) (Table 2).

The regrowth age factor (Table 2) caused significant effects ($p<0.05$) on total protein, albumin, and gamma-glutamyl transpeptidase with values greater than 12 months (6.01 g/dl; 3.06 g/dl and 24.50 IU/L, respectively) with respect to 4 and 8 months and the opposite occurred with total bilirubin, alanine aminotransferase and aspartate aminotransferase, where it was observed that the values decreased (0.28 to 0.15 mg/dl; 54.03 to 34.52 IU/L and 80.67 to 56.94 IU/L, respectively) with increasing regrowth age and alkaline phosphatase (130.23 IU/L) showed no differences ($p>0.05$).

The factor inclusion levels (Table 2) did not affect the serum levels of total protein (5.80 g/dl), albumin (2.91 g/dl), alkaline phosphatase (130.23 IU/L), and gamma-glutamyl transpeptidase (22.28 IU/ L) ($p>0.05$). There were significant differences ($p<0.05$) for total bilirubin, alanine aminotransferase, and aspartate aminotransferase, which decreased as the percentage of inclusion increased (0.25 to 0.18 mg/dl, 46.76 to 35.75 IU/L and 75.53 to 57.66 IU/L, respectively).

Table 2. Serum levels in guinea pigs fed pisonay meal (*Erythrina edulis*).

	Treatments										EEM	Probability			
	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9					
		Regrowth age (months)													
		4			8			12				T	Ed	I	EdxI
		Inclusion (%)													
		10	20	30	10	20	30	10	20	30					
TP(g/dL)	5.42	5.51	5.52	5.77	5.74	5.76	5.91	5.87	5.94	6.21	0.06	NS	*	NS	NS
ALB(g/dL)	3.03a	2.80b	2.83b	2.88a	2.81b	2.84a	2.91a	3.06a	3.05a	3.06a	0.01	**	**	NS	NS
TB(mg/dL)	0.17a	0.32b	0.26b	0.26b	0.23b	0.20a	0.19a	0.19a	0.15a	0.11b	0.00	**	**	**	NS
ALT(UI/L)	35.93a	64.22b	51.46b	46.41a	38.26a	36.12	31.00a	37.79a	35.93a	29.84a	1.30	**	**	**	NS
AST(UI/L)	63.94a	85.37b	81.76b	74.87a	75.21a	59.82a	49.30b	66.02a	56.01a	48.79b	1.65	**	**	**	NS
AP(UI/L)	118.16	137.59	132.89	130.94	134.23	131.73	126.98	131.63	124.84	121.18	2.31	NS	NS	NS	NS
GGT(UI/L)	20.75	19.58	20.32	21.42	21.10	21.52	23.01	24.03	24.60	24.88	0.38	**	**	NS	NS

SEM: standard error of the mean. a, b: Letters in the rows denote statistical difference of means.
T: Treatments. Ed: Age of regrowth (months). I: Inclusion of EELM (%). EdxI: Interaction Age of regrowth x Inclusion of EELM. NS: Not significant. *: $p<0.05$. **: $p<0.01$.

DISCUSSION

The serum protein levels found were close to the maximum limit and albumin showed a slight increase, without differing from the normal values reported for TP: 4.8 to 5.6 g/dl and ALB: 2.2 to 3.2 g/dl in male guinea pigs. In this strain, 13/N no clinical reference intervals have established been for the clinical chemistry parameters, where it observed was that the protein increases as the guinea pigs grew during the first 300 days and the albumin decreases with age (15). Total bilirubin decreased due to age and inclusion of EELM but was similar in the range of 0.0 to 1.0 mg/dL (16).

The differentiation of the serum metabolites was probably due to the inclusion of pisonay, as occurred when including 7, 14, 21, and 28% of *Erythrina* leaf meal in the diet for guinea pigs, which caused the variation and increase in the total protein of 5.07 to 6.50 g/dl and albumin from 3.88 to 4.71 g/dl (17), in another study, it is mentioned that the consumption of unconventional fodder such as *Lantana camara* in guinea pigs decreased total protein from 6.60 to 4.05 g/dl and total bilirubin from 1.01 to 0.49 mg/dl (18), the latter, without differing from the normal value for guinea pigs, to indicate the probable hepatoxicity caused by the inclusion of EELM, the presence of liver dysfunction would be observed, which would cause a decrease in the serum levels of TP and ALB since both are markers of the biosynthetic capacity of the liver (19).

This would indicate that the use of EELM in the diet for guinea pigs would not cause alterations in liver tissue and that it would depend on the amount in the food, which would cause a negative effect on liver function, evidenced by higher activities of aspartate aminotransferase and lower levels of albumin (20), variations in total protein would be primarily due to decreased albumin production due to liver disease, (21) and these alterations would be corroborated by the presence of severe hypoalbuminemia (10), in addition, intrahepatic and extrahepatic obstruction of bile flow and increased bilirubin level (22), also, another of the effects that would increase total serum protein values is the protein quantity and quality of the diet (23) and from a metabolic point of view, the serum protein would be concatenated with the digestible protein, which could decrease due to the moderate to the abundant presence of tannins in the food (24).

The enzymatic activity of transaminases tended to decrease as a result of regrowth age and inclusion of EELM, and both enzymes were similar to the ranges suggested by Genzer et al, who reported levels of 24 to 67 IU/L and 27 to 162 IU /L respectively (15). In this strain, increases in ALT we´re revealed as the guinea pigs grew (age, 0 to 150 d), males have a consistently higher ALT than females, but this trend isn´t seen with AST, these trends were strongest in adults (age, 151 through 900 d). The decreasing concentration of these enzymes due to the inclusion of pisonay meal in the integral food for guinea pigs would cause manifest chronic necrosis due to toxic substances (25), such as tannins and alkaloids reported in fresh pisonay leaves (6). This behavior was different, as was observed with the shrubby legume *Caesalpinia bonduc*, which has increased 9-fold aspartate aminotransferase due to hepatocyte injury related to acute intoxication (26). Another problem that would cause high concentrations of aminotransferases is intrahepatic cholestasis and liver injury (18), also, the limitation of the percentage of inclusion in the integral food for guinea pigs should be taken into consideration, which would avoid mild to moderate toxicity (27), and health problems as occurred in birds (28) and in small ruminants (29).

The probable cause of the decrease in aminotransferases is the existence of secondary compounds, which could cause negative effects on the nutrients in the feed and on animal health (30). These effects may be due to the number of tannins and saponins that would possibly cause negative effects in the inputs for animal feed (31). The alkaloids would cause acute intoxication and at the same time severe liver damage, the possible interactions of pyrrolizidine alkaloids (PAs) with nuclear receptors as a toxicological mode of action they not known so far, and in food products, PAs occur as complex mixtures of structurally different compounds (32). The aminotransferases, when decreased by the factors studied, would probably occur due to the increase in secondary metabolites as a result of the regrowth age (33), another factor is the presence of tannins that can induce aminotransferases and alkaline phosphatase to a detoxification process and that would cause negative effects on liver function (24).

The serum levels of alkaline phosphatase due to the effect of pisonay meal increased without differing from the guinea pigs that consumed the

control diet. The data found were comparable to the range of 29 to 205 IU/L indicated for adult male guinea pigs (34) and was contradictory to the values reported (16 to 83 IU/L) by Genzer et al (15). In this strain, ALP (0 to 184 d) showed high levels in growing animals and seems to reach levels similar to those in adults at 6 months of age. The variations found in guinea pigs may be due to non-harmful amounts of pisonay meal, to observe any disorder in the functional capacity of the liver, high toxic doses are required that would cause necrosis, inflammatory infiltration, hemorrhages, and congestion (35). The factors that can vary the increase in alkaline phosphatase in other animals were the addition of corn, sorghum, and millet as energy sources in the whole food that would cause the presence of gallstones (36), and the amount of phosphorus included in the diet or by the presence of phytates (37).

The gamma-glutamyl transpeptidase does not differ from the values found in guinea pigs that we´re fed with the control diet, but they increased due to the effect of regrowth age. The reported values are within the reference values obtained by Genzer et al (15), where GGT was observed to drop suddenly after 1 month before rising steadily within the adult range (2.5 to 21 U/L). This behavior has been observed with the addition of fresh foliage of *Moringa oleifera* as a protein source and antioxidant in replacement of concentrated feed (29), variations in the metabolite could show that it may be the protective mechanism of cells against toxicity (38).

In conclusion, the inclusion of EELM from three regrowth ages in the diet for guinea pigs influenced the levels of total bilirubin and aminotransferases, which decreased with increasing regrowth age and inclusion percentage. Liver metabolites are within acceptable levels for guinea pigs.

Conflict of interests

The authors express no conflict of interest.

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