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Article

# Effect of including pisonay (*Erythrina edulis*) meal on the hematological profile in guinea pigs (*Cavia porcellus*)

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**Abstract:**

This study aimed to evaluate the hematological profile of guinea pigs supplemented with *Erythrina edulis* leaf meal. *Erythrina edulis* leaves were collected at the regrowth ages of 4, 8, and 12 mo (A4, A8, A12), ground into meal, and included in the diets for guinea pigs at 10, 20 and 30 % (P10, P20, P30). A total of 80 improved and weaned male guinea pigs were randomly distributed for each diet. After 56 d, blood was collected directly from the jugular vein in EDTA tubes to analyze erythrocytes, mean corpuscular volume, hematocrit, hemoglobin, leukocytes, platelets, and mean platelet volume. Erythrocytes and hemoglobin were similar between diets. The factors of age and meal inclusion did not affect the values found in erythrocytes, mean corpuscular volume, hematocrit, and hemoglobin. The age factor (A4:8.55; A8:11.92; A12:10.14 x$10^3$/ul) and meal inclusion (P20:11.11; P30:12.08; P10:7.43 x $10^3$/uL) caused differences in leukocytes. Platelets were affected by the age factor (A4:391.98; A12:400.67; A8:467.08 x $10^3$/uL) and meal inclusion factor (P10:444.22; P20:443.05; P30:372.45 x $10^3$/uL). The mean platelet volume showed variations due to the age factor (A8:11.39; A12:11.31; A4:11.90 fL). *Erythrina edulis* leaf meal in guinea pig diets has potential as a feed input without altering the hematological profile, which would indicate that age and inclusion factors would not cause toxicity.

**Keywords:** Erythrocytes, Hematocrit, Hemoglobin, Leukocytes, Platelets.

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# Introduction

Guinea pig production systems in the Peruvian Sierra have changed from the family system, characterized by native guinea pigs fed on fodder, weeds, kitchen and harvest residues, which led to slow growth and the consumption or sale of guinea pigs at an age of 6 mo with average weights of 966 g(1), and the systems currently used are family-commercial and commercial, which have increased the production of guinea pigs by using improved guinea pigs, preferably the Peruvian breed, which receive mixed or complete foods, guinea pigs aged 30 to 72 d reached weights greater than 1,070 g(2); in addition, guinea pig meat is recognized as a good quality exotic meat due to its low amount of lipids and its use in gastronomic traditions(3).

The versatile feeding of guinea pigs leads to the search for alternative foods to increase productivity; for example, the inclusion levels of *Arachis pintoi* meal in 5, 10, and 15 % to replace alfalfa hay in the guinea pig diets for 49 d lead to average final weights of 997 g and 74 % carcass yield(4); likewise, the supplementation with tarwi meal in 18 % in the complete concentrate feed caused a positive response in body weight gain(5). Another alternative is the use of tropical forage plants; *Erythrina poeppigiana* plus 15 g of feed for 56 d in the production process resulted in 8 g of daily weight gain and 66 % of carcass yield(6). *Erythrina* sp. (pisonay), when included up to 50 % as fresh forage in the fattening phase of guinea pigs, allowed increasing body weight up to 1,221 g(7). Furthermore, an increase in serum aminotransferase concentrations, a greater presence of liver pathologies, and a decrease in the liver/body weight ratio were observed with the inclusion of 50 and 100 % pisonay(8); likewise, high serum creatinine and urea levels can cause disorders in the renal function of guinea pigs(9). In rabbits, it has been observed that including *Moringa oleifera* meal causes variations in hematology(10).

The hematological profile in animals is performed to know the state of health and physiological variations(11) and to assess the toxicity caused by food consumption(12); it is mentioned that plants and additives in feed inputs present toxic agents that would cause hemolytic anemia due to a decrease in erythrocytes, this behavior would help to diagnose diseases related to metabolism or eating disorders(13), as occurred with the toxicosis of *Ipomoea carnea* in guinea pigs, which caused normocytic hypochromic anemia due to a significant reduction in erythrocytes, hematocrits, and hemoglobin concentration after 20 d and non-regenerative anemia at 40 d(14); in another study, the inclusion of *Curcuma longa* powder in guinea pig diets had a variable influence on the concentration of leukocytes, lymphocytes, and monocytes, which was caused by stimuli in the immune system and antioxidant effects[15]. The present experiment aimed to assess the effect of including pisonay (*Erythrina edulis*) meal on the hematological parameters of guinea pigs (*Cavia porcellus*).

## Material and methods

Foliage was collected from trees used in living fences and animal feeding from the sector located in Mosoccpampa, district of Tamburco, located at 2,880 m asl. Pisonay trees were selected for convenience; the foliage was cut after 4, 8, and 12 mo of regrowth; after drying the leaves in the shade for approximately 30 d, the meal was made in a hammer mill with a 2 mm diameter sieve.

Subsequently, the complete diets (Table 1) were processed into meal, considering 18 % protein and 3,000 kcal of digestible energy/kg of dry matter, and the diets were named D0, D1, D2, D3, D4, D5, D6, D7, D8, and D9; each diet was added 10 % (P10), 20 % (P20), and 30 % (P30) of pisonay meal for each age of regrowth: 4 mo (A4), 8 mo (A8), and 12 mo (A12), and a control diet (D0) with the inclusion of 20 % alfalfa meal.

The experimental complete diets were as follows:
D0: Complete diet, it includes 20 % alfalfa meal
D1: Complete diet, it includes 10 % meal of pisonay with a regrowth age of 4 mo
D2: Complete diet, it includes 20 % meal of pisonay with a regrowth age of 4 mo
D3: Complete diet, it includes 30 % meal of pisonay with a regrowth age of 4 mo
D4: Complete diet, it includes 10 % meal of pisonay with a regrowth age of 8 mo
D5: Complete diet, it includes 20 % meal of pisonay with a regrowth age of 8 mo
D6: Complete diet, it includes 30 % meal of pisonay with a regrowth age of 8 mo
D7: Complete diet, it includes 10 % meal of pisonay with a regrowth age of 12 mo
D8: Complete diet, it includes 20 % meal of pisonay with a regrowth age of 12 mo
D9: Complete diet, it includes 30 % meal of pisonay with a regrowth age of 12 mo

From 80 improved weaned male guinea pigs approximately 15 d-old, 8 guinea pigs were randomly distributed for each experimental diet, two replications per diet and 4 guinea pigs per replication were assigned; the guinea pigs were previously identified with numbered metal ear tags. The guinea pigs were placed in single-level mesh cages, with capacity for four guinea pigs, with dimensions of 0.9 x 0.9 x 0.4 m.

**Table 1:** Complete diets (%) used in the experiment

| Inputs | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 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 |
| Soybean cake | 18.4 | 17.3 | 16.2 | 14.5 | 17.3 | 16.2 | 14.3 | 17.4 | 16.1 | 14.6 |
| Corn | 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 |
| Mycotoxin sequestrant | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Vitamins and minerals | 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 |

The guinea pigs received the complete diets for 56 d in hopper-type feeders and a 7-d adaptation phase was considered; in addition, each cage had bell-type drinkers where fresh water was added at will.

Once the experimental phase was completed, the blood sample was obtained. For the blood count, blood was collected directly from the jugular vein in EDTA tubes (1 mL). Two readings of the erythrocyte count (RBC), mean corpuscular volume (MCV), hematocrit (HCT), hemoglobin (Hb), leukocytes (WBC), platelets (Plat), and mean platelet volume (MPV) were taken with the automatic hematology analyzer (Urit 2900 Vet Plus, China). The differential count of leukocytes – neutrophils (N), lymphocytes (L), monocytes (M), and eosinophils (E) – was performed in duplicate by blood smear in 100 cells from each sample.

Data from the 10 diets were analyzed using a completely randomized design. The factors of age and meal inclusion were evaluated through the 3 x 3 factorial arrangement without considering the control diet (D0); the normality test was performed using the Shapiro-Wilk test and to compare the means, the homogeneity of variances test was previously applied through the Levene test. A significance level of 0.05 was considered. RBC, HCT, Hb, WBC, Plat, and MPV data were transformed by Arcsine. In addition, for the data found in M and E, the square root transformation $(Y + 0.5)^{1/2}$ was used.

## Results and discussion

Erythrocytes (Table 2) were similar between diets and the factors of age and meal inclusion did not affect the values found, which ranged from 5.52 to 5.96 x $10^6$/uL ($P$>0.05). Regarding mean corpuscular volume, the D3, D5, D6, D7, D8, and D9 diets were different from the D0 diet (control diet) ($P$<0.05) and the factors of age and meal inclusion did not influence the indicators found, which remained between 71.06 and 72.14 fL ($P$>0.05).

The erythrocytes follow trends similar to those reported in the reference intervals indicated for 13/N(16), Dunkin-Hartley(17), and native guinea pigs(18). The mean corpuscular volume was similar to the report in 13/N guinea pigs(16). Djoumessi *et al*(15) mention that the decrease in erythrocytes would be related to inadequate feeding.

The hematocrit (Table 2) showed that D3 (42.99 %) was similar to D0 (44.98 %) and regarding the rest of the diets, they were different and decreased to 40.88 % ($P$<0.05); the factors of age and meal inclusion did not affect the indicators found, which were between 41.19 and 42.50 % ($P$>0.05).

The hematocrit was close to the minimum reference value indicated for 13/N(16) and Dunkin-Hartley(17) guinea pigs, unlike D0, which was similar to that reported in native guinea pigs(18); this behavior has a direct relationship with the adequate amount and intake of water during the experiment.

The concentration of hemoglobin (Table 2) was similar between the diets and ranged from 14.11 to 14.96 g/dL ($P$>0.05); the factors of age and meal inclusion would not affect the indicators found, which were between 14.47 and 14.72 g/dL ($P$>0.05). Hemoglobin is similar to the reference intervals indicated for 13/N(16), Dunkin-Hartley(17), and native guinea pigs(18).

Leukocytes (Table 2) showed similarity between diets, except for D2, D4, and D7, which were different from D0 ($P$<0.05); the age factor would cause differences between A4 (8.55 x $10^3$/uL) and A8 (11.92 x $10^3$/uL) and they were similar to A12 (10.14 x $10^3$/uL) ($P$<0.05); the meal inclusion factor induced a similarity between P20 (11.11 x $10^3$/uL) and P30 (12.08 x $10^3$/uL) and it decreased due to the effect of P10 (7.43 x $10^3$/uL) ($P$<0.05).

The percentage of neutrophils (Table 2) was affected by the D3 and D4 diets, which were lower and different from the D0 diet ($P$<0.05). In lymphocytes, there were differences between D4 and D0 ($P$<0.05); in monocytes, D3, D6, D7 and D9 were different from D0 ($P$<0.05); and eosinophils showed differences between D6 and D9 when compared with D0 ($P$<0.05).

The age factor caused differences and an increase in neutrophils between A4 (43.31 %), A8 (50.20 %), and A12 (58.12 %) ($P$<0.05); in lymphocytes, a decrease was observed as the age of regrowth of A4 (51.45 %), A8 (44.85 %), and A12 (36.72 %) ($P$<0.05) increased; monocytes and eosinophils showed no differences ($P$>0.05) and remained between 2.93 to 3.27 % and from 0.85 to 1.27 %, respectively.

In neutrophils, the factor of meal inclusion induced a similarity between P20 (52.48 %) and P30 (53.93 %), which differed from P10 (45.22 %) ($P$<0.05); lymphocytes in P10 (49.06 %) were higher than P20 (42.46 %) and P30 (41.51 %), which were similar ($P$>0.05).

Monocytes and eosinophils showed similarity between P10 (2.88 and 0.64 %) and P20 (2.56 and 0.91 %), both indicators were increased by P30 (3.91 and 1.62 %, respectively) ($P$<0.05).

The number of leukocytes covered a heterogeneous amplitude compared to the references mentioned for 13/N[(16)] and Dunkin-Hartley[(17)] guinea pigs, unlike guinea pigs of the meat and early lines, which exhibited values from 3.47 to 14.94 x $10^3$/uL[(19)]. These variations observed by the addition of pisonay meal in the complete feed for guinea pigs would not cause harmful consequences, as occurred with the inclusion of *Mucuna utilis* in the diet of rabbits, it did not cause disorders in hematological parameters[(20)]; a similar trend was observed with *Azadirachta indica*, which was not harmful for hematopoiesis[(21)] and it turns out to be contradictory with the leaf meal of *Morinda lucida* as an antimicrobial supplement in the diet for chickens, it stimulated the decrease in leukocytes[(22)].

The percentages of neutrophils, lymphocytes, monocytes, and eosinophils follow trends similar to the reference intervals indicated for 13/N[(16)] and Dunkin-Hartley[(17)] guinea pigs. Regarding monocytes and eosinophils, they showed similarity to the values reported for native guinea pigs[(18)]. Zimmerman *et al*[(23)] mention that toxicity would increase the number of neutrophils and their maturation would be accelerated. The stable percentages of leukocytes, lymphocytes, and monocytes would indicate the absence of inflammatory and infectious diseases in guinea pigs[(24)].

Pisonay meal would not cause an increase in neutrophils and eosinophils, therefore, the immune system would not be activated since pisonay would have a minimum amount of toxic compounds; this behavior was observed with the addition of meal from *Agave tequilana* stems in rabbits[(25)]; in addition, eosinophilia is an indicator of allergies and inflammatory processes, which were not observed in the guinea pigs; the addition of natural supplements as growth promoters in Broiler chickens preserved the normal percentage of eosinophils[(26)].

Platelets (Table 2) showed similarity between diets, except for D5, which was different from D0 ($P$<0.05); the age factor would cause similarity between A4 (391.98 x $10^3$/uL) and A12 (400.67 x $10^3$/uL), which were lower than A8 (467.08 x $10^3$/uL) ($P$<0.05), and the meal inclusion factor induced a similarity between P10 (444.22 x $10^3$/uL) and P20 (443.05 x $10^3$/uL) and it decreased due to the effect of P30 (372.45 x $10^3$/uL) ($P$<0.05).

The mean platelet volume (Table 2) in D6 and D7 differed from the D0 diet ($P<0.05$); the age factor produced similarity between A8 (11.39 fL) and A12 (11.31 fL), which decreased compared to A4 (11.90 fL) ($P<0.05$); and the meal inclusion factor induced similarity in the values found, which ranged from 11.42 to 11.63 fL ($P>0.05$).

Platelets were similar to the references mentioned for Dunkin-Hartley guinea pigs(17), except for D5, the effect of A8 and the effects of P10 and P20 were above the maximum value indicated for 13/N(16) guinea pigs; these variations would indicate that the complete diet for guinea pigs could induce chronic diseases(27). The mean platelet volume reported in all cases was above the values reported for 13/N(16) and Dunkin-Hartley(17) guinea pigs. These variations could be a consequence of the altitude level; this was corroborated with values from 249 to 800 x $10^3$/uL obtained in guinea pigs of the meat and early lines that were raised at 3,350 m altitude(19).

Nutrition is one of the factors influencing the hematological profile(28); in the leaves of *Erythrina edulis*, there was a notable presence (++) of alkaloids, flavonoids, and saponins, and a mild presence (+) of sterols(29); in another study, in two phenological stages, the antinutritional levels were below 2 % on a dry matter basis(30); this would indicate the slight variation observed in some hematological indicators due to the effect of pisonay meal and would probably not represent a nutritional problem in guinea pigs. On the other hand, if one or more blood components are directly affected, it would be related to primary hematotoxicity, which will depend on the amount and time of exposure to an extrinsic substance(31).

## Conclusions and implications

*Erythrina edulis* leaf meal in guinea pig diets has potential as a feed input without altering the number of erythrocytes, mean corpuscular volume, percentage of hematocrits, hemoglobin, leukocytes, platelets, and mean platelet volume, which would indicate that age and inclusion factors would not cause toxicity.

**Table 2:** Influence of pisonay meal on the hematological profile of guinea pigs

| **Parameters** | | **D0** | **D1** | **D2** | **D3** | **D4** | **D5** | **D6** | **D7** | **D8** | **D9** | **SEM** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RBC, $10^6$/uL | M | 5.92$^{a}$ | 5.80$^{a}$ | 5.88$^{a}$ | 5.96$^{a}$ | 5.86$^{a}$ | 5.70$^{a}$ | 5.64$^{a}$ | 5.89$^{a}$ | 5.88$^{a}$ | 5.52$^{a}$ | 0.03 |
| | m | 5.89 | 5.81 | 5.88 | 5.97 | 5.73 | 5.73 | 5.63 | 5.89 | 5.85 | 5.49 | |
| MCV, fL | M | 75.25$^{a}$ | 72.81$^{a}$ | 71.91$^{a}$ | 71.69$^{b}$ | 72.48$^{a}$ | 71.70$^{b}$ | 71.51$^{b}$ | 69.98$^{b}$ | 71.68$^{b}$ | 71.51$^{b}$ | 0.29 |
| | m | 75.63 | 72.08 | 73.13 | 72.38 | 73.50 | 72.08 | 72.10 | 70.15 | 71.88 | 72.23 | |
| HCT, % | M | 44.98$^{a}$ | 41.62$^{b}$ | 41.56$^{b}$ | 42.99$^{a}$ | 41.61$^{b}$ | 41.29$^{b}$ | 40.98$^{b}$ | 41.16$^{b}$ | 40.88$^{b}$ | 41.54$^{b}$ | 0.22 |
| | m | 44.95 | 41.60 | 42.25 | 43.15 | 40.98 | 41.30 | 41.33 | 41.73 | 41.00 | 42.13 | |
| Hb, g/dL | M | 14.53$^{a}$ | 14.54$^{a}$ | 14.11$^{a}$ | 14.76$^{a}$ | 14.58$^{a}$ | 14.62$^{a}$ | 14.52$^{a}$ | 14.96$^{a}$ | 14.88$^{a}$ | 14.32$^{a}$ | 0.08 |
| | m | 14.63 | 14.58 | 15.08 | 14.60 | 14.48 | 14.58 | 14.53 | 14.95 | 14.83 | 14.30 | |
| WBC, $10^3$/uL | M | 12.23$^{a}$ | 8.98$^{a}$ | 7.15$^{b}$ | 9.52$^{a}$ | 6.22$^{b}$ | 13.63$^{a}$ | 15.93$^{a}$ | 7.10$^{b}$ | 12.55$^{a}$ | 10.76$^{a}$ | 0.52 |
| | m | 12.63 | 9.05 | 7.73 | 9.55 | 5.85 | 15.48 | 14.38 | 6.78 | 12.53 | 10.60 | |
| N, % | M | 52.50$^{a}$ | 44.25$^{a}$ | 45.88$^{a}$ | 39.81$^{b}$ | 38.18$^{b}$ | 51.31$^{a}$ | 61.12$^{a}$ | 53.25$^{a}$ | 60.25$^{a}$ | 60.88$^{a}$ | 1.22 |
| | m | 52.85 | 44.35 | 42.85 | 43.85 | 36.85 | 49.50 | 64.35 | 52.55 | 61.35 | 61.35 | |
| L, % | M | 45.00$^{a}$ | 50.38$^{a}$ | 49.62$^{a}$ | 54.38$^{a}$ | 57.43$^{a}$ | 42.38$^{a}$ | 34.73$^{b}$ | 39.38$^{a}$ | 35.38$^{b}$ | 35.43$^{a}$ | 1.20 |
| | m | 45.50 | 49.00 | 53.50 | 51.50 | 58.00 | 42.00 | 32.25 | 38.50 | 33.00 | 35.50 | |
| M, % | M | 1.31$^{a}$ | 2.18$^{a}$ | 3.00$^{a}$ | 3.62$^{b}$ | 2.68$^{a}$ | 2.68$^{a}$ | 4.06$^{b}$ | 3.75$^{b}$ | 2.00$^{a}$ | 4.06$^{b}$ | 0.18 |
| | m | 1.25 | 1.75 | 2.50 | 4.25 | 2.50 | 2.00 | 3.75 | 3.75 | 1.75 | 4.00 | |
| E, % | M | 0.43$^{a}$ | 0.43$^{a}$ | 0.75$^{a}$ | 1.38$^{a}$ | 0.56$^{a}$ | 1.00$^{a}$ | 1.62$^{b}$ | 0.93$^{a}$ | 1.00$^{a}$ | 1.88$^{b}$ | 0.10 |
| | m | 0.50 | 0.25 | 0.50 | 1.00 | 0.25 | 0.50 | 1.50 | 1.00 | 0.50 | 1.50 | |
| Plat, $10^3$/uL | M | 393.35$^{a}$ | 383.40$^{a}$ | 437.70$^{a}$ | 354.85$^{a}$ | 507.35$^{a}$ | 527.54$^{b}$ | 366.34$^{a}$ | 441.92$^{a}$ | 363.91$^{a}$ | 396.18$^{a}$ | 11.12 |
| | m | 392.75 | 351.85 | 431.70 | 359.48 | 527.83 | 533.50 | 365.50 | 424.78 | 360.25 | 398.73 | |
| MPV, fL | M | 12.68$^{a}$ | 11.74$^{a}$ | 12.05$^{a}$ | 11.93$^{a}$ | 11.54$^{a}$ | 11.40$^{b}$ | 11.25$^{b}$ | 11.00$^{b}$ | 11.45$^{b}$ | 11.48$^{b}$ | 0.10 |
| | m | 12.40 | 11.58 | 11.63 | 11.70 | 11.43 | 11.38 | 11.00 | 10.95 | 11.33 | 11.40 | |

RBC=erythrocytes; MCV= mean corpuscular volume; HCT= hematocrit; Hb= hemoglobin; WBC= leukocytes; N= neutrophils; L= lymphocytes; M= monocytes; E= eosinophils; Plat= platelets; MPV= mean platelet volume; M= mean; m= median; SEM= standard error of the mean.

$^{ab}$ Different letters in the rows indicate significant difference in means ($P \leq 0.05$).

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