



Hematological and Performance Parameters Trend in Steers and Heifers in Feed Lot and Supplemented with Palm Oil (*Elaeis guineensis* Jack)

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Article History: 22-669

Received: 24-Jul-22

Revised: 14-Sep-22

Accepted: 16-Sep-22

ABSTRACT

This study compares the daily weight gain (ADG) and the hematological profile of cattle in feed lot and supplemented with palm oil (POil). Overall, 20 male and 24 female Holstein-Cebu (5/8-3/8) cattle with an average weight of 249±43 kg were used. Three groups of eight animals each were formed with females, which were housed and received 0, 2, and 4% POil in the diet (FSWO, FS2O, FS4O). With males, only two groups were formed, one group that kept grazing and received 2% POil (MG2O, n=8) and one group stabled and received 4% POil (MS4O, n=12). Peripheral blood was obtained to determine the hematocrit (HCT), plasma protein concentration (TPP, g/dL), and eosinophil count (EOS), and a complete blood sample was processed in an automatic analyzer to determine the erythron and platelet formula. Analysis of repeated measures over time was performed. Stabled steers showed the highest ADG (1.5kg/d), whereas females received 2 or 4% oil had similar ADG levels (1.3 to 1.4kg/d) than grazing steers. At the first sampling, EOS counts were 1.5 x 10³ cells/μL of blood, but they were reduced to 0.23 x 10³ cells/μL of blood at the end of the study. The lowest HCT was obtained in grazing steers (32.8%). It is concluded that in stabled steers, the red formula is improved, and the EOS count reduced. Palm oil supplementation generated the highest daily weight gain in stabled steers.

Key words: Cattle, Daily Gain, Eosinophils, Hematocrit, Hemoglobin.

INTRODUCTION

Mexico is the sixth largest producer of beef worldwide (FAOSTAT 2022), raising livestock in all ecosystem types of the country at different technological levels (Greenwood 2021). At a total area of 108.9 million hectares (Enríquez-Quiroz et al. 2021), 35.6 million head of cattle, 12,825 metric tons of milk, and 2.1 million tons of meat are produced annually (SIAP 2020). The dry and humid tropical zones of Mexico have a high importance in the national bovine production, accounting for 18% of the total milk production and 40% of the meat production (Enríquez-Quiroz et al. 2021). Their systems are characterized by the use of *Bos indicus* breeds grazing in C4-type pastures (Greenwood 2021). In these regions, grazing represents the most economical means of transforming low-quality pastures into foods of high nutritional value.

However, cattle production has several limitations in the tropics due to environmental changes, which cause fluctuations in animal performance because of the variation in forage production throughout the year. Climatic factors directly influence the production and nutritional value of the forage produced (dos Santos et al. 2019). In addition, variable environmental conditions also cause heat stress and favor the development of parasites and diseases, damaging animal health (de Almeida Camargo and Pereira 2022). Therefore, the productive indicators are generally low, with weight gains of less than 650g per day (Sossa and Barahona 2015) and with a high prevalence of external (flies and ticks) and internal parasites (gastrointestinal nematodes and trematodes), among which *Fasciola hepatica* and paramphistomids stand out, all of them considered endemic to warm regions in Mexico (Ico-Gómez et al. 2021).

Cite This Article as: Flores-Santiago EDJ, González-Garduño R, Sosa-Pérez G, Villa-Mancera A and Córdova-Pérez C, 2022. Hematological and performance parameters trend in steers and heifers in feed lot and supplemented with palm oil (*Elaeis guineensis* Jack). International Journal of Veterinary Science x(x): xxxx. <https://doi.org/10.47278/journal.ijvs/2022.206>

In addition to the high economic costs for parasite control and the negative impact on productivity (Rodríguez-Vivas et al. 2017), the hematological profiles of the animal are affected, which tend to present a high variability due to multiple factors. Among these, nutritional deficiencies stand out, especially when there are mineral deficiencies, among which the most important ones are micro-mineral deficiencies. They are also modified with the level of production and the presence of diseases (Ruginosu et al. 2010), as well as by genetic and environmental factors such as age (de Lima et al. 2015), breed, sex, season (Purwar et al. 2019), physiological state (pregnancy and lactation), and others.

Hematological analysis is relevant in diagnosing anemia-related diseases and is also useful in diagnosing many other systemic diseases. However, the diagnosis of a disease can not only be based on the blood count, which can provide valuable information in the diagnosis and the formulation of a prognosis (Roland et al. 2014). The first step in determining whether an animal has hematologic abnormalities is a comparison of the hematologic results to a set of reference values, normal values, or reference ranges of healthy animals (George et al. 2010). However, as the values of the hematological profile in cattle are dependent on many factors, it is important to evaluate these profiles under particular conditions and, above all, to know the evolution of these elements after a change in management conditions, such as when the animals are stabled for finishing. In this sense, during cattle fattening, feed consumption modifies the hematological profiles, making it important to evaluate the impact of products such as palm oil (POil) on the hematological profile.

POil is composed of saturated (SAFA; 48%) and unsaturated (UFA; 52%) fatty acids. Palmitic acid (85%) and oleic acid (88%) are the most abundant SAFA and UFA, respectively (Gesteiro et al. 2019), and POil oil is also rich in A and E vitamins. Due to the high amount of energy POil has been used in animal feed for several years to improve quality of carcass (Kang et al. 2011) and currently milk production (Frank et al. 2022) and feed conversion ratio (FCR) without any adverse effects on rumen fermentation (Matsuba et al. 2019). The by-products resulting from the oil extraction process have been used in animal feed (Tomkins and Drackley 2010), achieving the replacement of cereals as the energy base of diets and opening new areas for the management of fatty acids in animal feed, with production yields comparable to those considered optimal (Ghani et al. 2017). Therefore, the objective of the study was to compare the daily weight gain and the changes in the hematological profiles of grazing and stabled cattle supplemented with POil.

MATERIALS AND METHODS

Ethical statement: All procedures were carried out in accordance with the Official Mexican Standard-062-Z00-1999, on the technical specifications for the production, care and use of laboratory animals.

Location: The experiment was carried out from March to June 2021 at the facilities of the experimental farm of the Southeast Regional University Unit (URUSSE) of the Autonomous University of Chapingo (UACH) in Teapa,

Tabasco, Mexico, located at 17° 34' 30" North latitude and 92° 56' 15" West longitude, at an elevation of 60 meters above sea level. The region has a warm humid climate with a rainy regime in summer. The average annual temperature is 27.8°C, with 3,863mm of rain per year (CONAGUA 2021).

For this research, 44 bovines (20 males and 24 females) of the Holstein-Zebu breed (5/8 Holstein-3/8 Zebu) were used, with an age of 3.5±0.4 years and an average weight of 249±43kg in males and 211±39kg in females. All animals were initially grazing in paddocks with *Urochloa brizantha*. At the beginning of the study, fecal and blood samples were taken to determine the parasitological and hematological state. The animals were then sprinkled with an acaricidal mixture consisting of chlorpyrifos and permethrin (Garra Ban Mo 29®, La Pisa SA de CV, Mexico) at the recommended dose and dewormed with doramectin (Flok doramectin 1.10%, Biogenesis Bagó, Argentina) applied at the dose recommended by the manufacturer.

Eight steers continued grazing and received a supplement with 2% POil (MG2O), and 12 steers were housed and received 4% POil in the diet (MS4O). With the females, three groups of eight animals each were formed, which were housed and received a control diet (FSWO) and two levels of POil in the ration: 2% (FS2O) and 4% (FS4O). The stabled animals were given 20 days to adapt to the diet, and later, all animals received the corresponding experimental diet (Table 1).

The housed animals were individually placed in a pen under 50% black agricultural shade mesh to reduce climatic stress; each pen was equipped with a plastic feeder and a drinker adapted to the facilities to provide water *ad libitum*. Food was provided twice a day, at 8 am and at 2 pm.

Animal weight: Every 15 days, all animals were weighed on a livestock scale model RG (Revolta Maza SA de CV, Mexico) with a sensitivity of 1kg. The ADG was estimated through the following formula (initial weight - final weight) / number of days.

Sampling: For the parasitological examination, monthly fecal samples were taken to determine the presence of internal parasites, using the McMaster technique with a sensitivity of 1:50 (Thienpont et al. 2003). A copro-culture was performed at the beginning of the study to determine the genera or species of nematodes (van Wyk and Mayhew 2013). The number of horn flies (*Haematobia irritans*) was also determined by counting via photographs (Maldonado-Simán et al. 2018).

The blood sample was taken from the coccygeal vein, using a vacutainer tube with EDTA as anticoagulant (Vacutainer; BD Biosciences, Franklin Lakes, NJ, USA). Using the complete blood sample, the hematocrit (HCT) was determined (Billett 1990). The total plasma protein concentration (TPP, g/dL) was determined by refractometry, and the peripheral eosinophil count (EOS) was determined as described in Dawkins et al. (1989). Blood glucose concentration was also measured with automatic equipment (accu-chek instant, Roche). A blood sample was processed in a hematology analyzer (Medonic CA 620/530 Vet, Brand Boule Medical AB, Stockholm, Sweden) to determine the erythron profile, measuring

hemoglobin (HGB), red blood cell (RBC), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW), and the platelet formula: platelets (PLT), plateletcrit (PTC), mean platelet volume (MPV), platelet distribution width (PDW).

Statistical design: The information analyzed corresponded to the productive and hematological indices of the cattle supplemented with POil. Analysis of variance was performed with a model of measures repeated over time, using the PROC MIXED procedure of SAS (SAS 2017):

$$Y_{ijk} = \mu + \zeta_i + \delta_{ij} + T_j + (\zeta * T)_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} =response variable (weight, ADG, hematologic variables), ζ_i =effect of i-esim treatment (I=FSWO, FS2O, FS4O, MS4O, MG2O), δ_{ij} =random error from animal,

T_j =effect of j-esim time (1, 15, 30, 45, and 60 días), $(\zeta * T)_{ij}$ = interaction between treatment and time, ε_{ijk} = experimental error associated with repeated measures.

RESULTS

At first sampling, a fecal count of trichostongylid eggs of 71 ± 167 and 332 ± 450 EPG of *Strongyloides papillosus* was observed. Of the nematode species, *Cooperia punctata* was the main species, and the presence of *S. papillosus* was confirmed in the infective larvae obtained from the copro-culture. Deworming was 97% effective, and in the following samplings the FEC values were less than 5 EPG on average. The initial horn fly counts were 117 ± 96 , and a reduction was observed in subsequent samplings to 17 ± 10 , although the presence of the house fly increased.

Table 1: Ingredients in the diet provided to growing cattle supplemented with palm oil.

Composition (% DM)	Female (heifers)			Male (Steers)	
	0% POil (FSWO)	2% POil (FS2O)	4% POil (FS4O)	4% POil (MS4O)	2% POil (MG2O)
Grass (<i>Urochloa brizantha</i>)	30	30	30	30	¥
Ground corn	57	55	53	53	76
Soybean paste	8	8	8	8	18
Molasses	2	2	2	2	2
Urea	1	1	1	1	0
Mineral premix (Vit+Min)	2	2	2	2	2
Palm oil	-	2	4	4	2
Chemical composition-% DM					
DM (%)	89.9	88.9	88.3	88.3	90.4
CP (%)	14.4	13.8	13.6	13.6	16.6
NDF (%)	43.5	43.5	40.8	40.8	39.8
ADF (%)	14.8	15.4	16.6	16.6	14.3
Ashes (%)	5.04	4.59	4.40	4.40	4.38
Gross Energy (Mcal/kg DM)	3.83	3.79	3.82	3.82	3.91

DM. Dry matter. CP. Crude protein. NDF. neutral detergent fiber. ADF. Acid detergent fiber. ¥ grazing in *Urochloa humidicola* paddocks.

Table 2: Hematological and productive values of steers of the Holstein-Zebu breed in each treatment.

Variable	Heifers (n=24)			Steers (n=20)		
	0% FSWO	2% FS2O	4% FS4O	4% MS4O	2% MG2O	Standard Deviation
Starting weight (Kg)	200	225	209	249	259	35.6
Final weight (Kg)	269	312	294	366	337	43.8
Average weight (Kg)	231.5 ^c	267.1 ^b	249.4 ^{bc}	300.7 ^a	295.5 ^a	45.4
ADG (Kg)	1.0 ^c	1.4 ^{ab}	1.3 ^b	1.5 ^a	1.3 ^b	0.3
TPP (g/dL)	7.7 ^{bc}	8.1 ^a	7.9 ^{ab}	7.9 ^{ab}	7.6 ^c	0.7
GLU (mg/dL)	75 ^a	68 ^b	70 ^{ab}	65 ^b	63 ^b	38.9
EOS (10 ³ /mL)	492 ^b	351 ^b	554 ^b	535 ^b	893 ^a	589
RBC (10 ⁶ /μL)	9.0 ^a	9.3 ^a	9.0 ^a	9.1 ^a	8.2 ^b	0.9
HCT (%)	35.0 ^a	35.8 ^a	34.6 ^a	35.7 ^a	32.8 ^b	3.3
MCV (fL)	39.5 ^a	39.3 ^a	39.3 ^a	39.9 ^a	40.9 ^a	3.6
RDW (%)	18.1 ^b	17.7 ^b	18.3 ^{ab}	18.9 ^a	16.5 ^c	1.6
RDW _a (fL)	29.0 ^{ab}	28.4 ^b	29.0 ^{ab}	30.0 ^a	28.9 ^{ab}	3.1
HGB (g/dL)	12.0 ^a	12.0 ^a	11.3 ^b	12.1 ^a	11.4 ^b	1.1
MCH (pg)	13.5 ^b	13.2 ^{bc}	12.7 ^c	13.5 ^b	14.1 ^a	1.1
MCHC (g/dL)	34.2 ^{ab}	33.7 ^b	32.5 ^c	33.7 ^b	34.6 ^a	1.8
PLT (10 ⁶ /mL)	252.2 ^a	243.6 ^{ab}	235.1 ^{ab}	202.1 ^b	104.7 ^c	98.0
PCT (%)	0.2 ^a	0.2 ^a	0.2 ^{ab}	0.1 ^b	0.1 ^c	0.1
MPV (fL)	6.9 ^{abc}	6.9 ^{ab}	7.0 ^a	6.8 ^{bc}	6.7 ^c	0.4
PDW (fL)	8.4 ^a	8.5 ^a	8.6 ^a	8.4 ^a	8.4 ^a	0.5

FSWO: Females stabled without palm oil, FS2O: Females stabled with 2% palm oil, FS4O: Females stabled with 4% palm oil, MS4O: Males stabled with 4% palm oil and MG2O: Grazing males with 2% palm oil. ADG, average daily gain, TPP total plasma protein, GLU Glucose, EOS eosinophils, RBC red blood cell count, HCT hematocrit, MCV mean cell volume, RDW distribution width of red blood cells, HGB hemoglobin, MCH: mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, PLT platelet, PCT plateletcrit, MPV mean platelet volume, PDW platelet distribution width.

Stabled steers fed with 4% POil showed the highest ADG (1.5kg/d). Females receiving 2 or 4% POil had similar weight gains (1.3 to 1.4kg/d) than grazing steers receiving 2% POil (Table 2).

Females without oil supplementation had the lowest ADG (1.0kg/d). The ADG increased in all groups of animals ($P<0.05$). The highest values were obtained in the males receiving 4% POil in diet (MS4O), with an ADG of 1.7kg/day at 60 days after the experiment had started. In the opposite case, the lowest weight gain (1.2 kg/day) was obtained in females without POil (FSWO) (Fig. 1).

Erythron

Differences were observed between stabled and grazing animals in the values of the erythron ($P<0.05$). Grazing steers had lower values of HCT, RBC, and HGB compared to the remaining animals (Table 2). Both females and stabled males maintained similar values of HCT and RBC, and the size of the red blood cells showed no differences among the five groups ($P>0.05$).

The behavior of the variables over time showed that HCT was similar for both male and female stabled cattle. In grazing animals (MG2O), the values were lowest ($P<0.05$). In all stabled groups, the hematological variables increased after 15 days of starting the experiment.

The glucose concentrations in each treatment were in the range of 40 to 88mg/dL. The heifers that received the oil-free diet (FSWO) showed the highest blood glucose concentrations, whereas the grazing animals (MG2O) had the lowest values. Between 15 and 20 days after starting the experiment, the highest glucose values were observed in all groups.

Eosinophils

In the first sampling, when the animals were grazing, the EOS counts were 1.5×10^3 cells/ μ L of blood, but over time, these values decreased to 0.23×10^3 cells/ μ L of blood. In addition, the stabled animals presented lower amounts in the first 30 days of housing (Fig. 3).

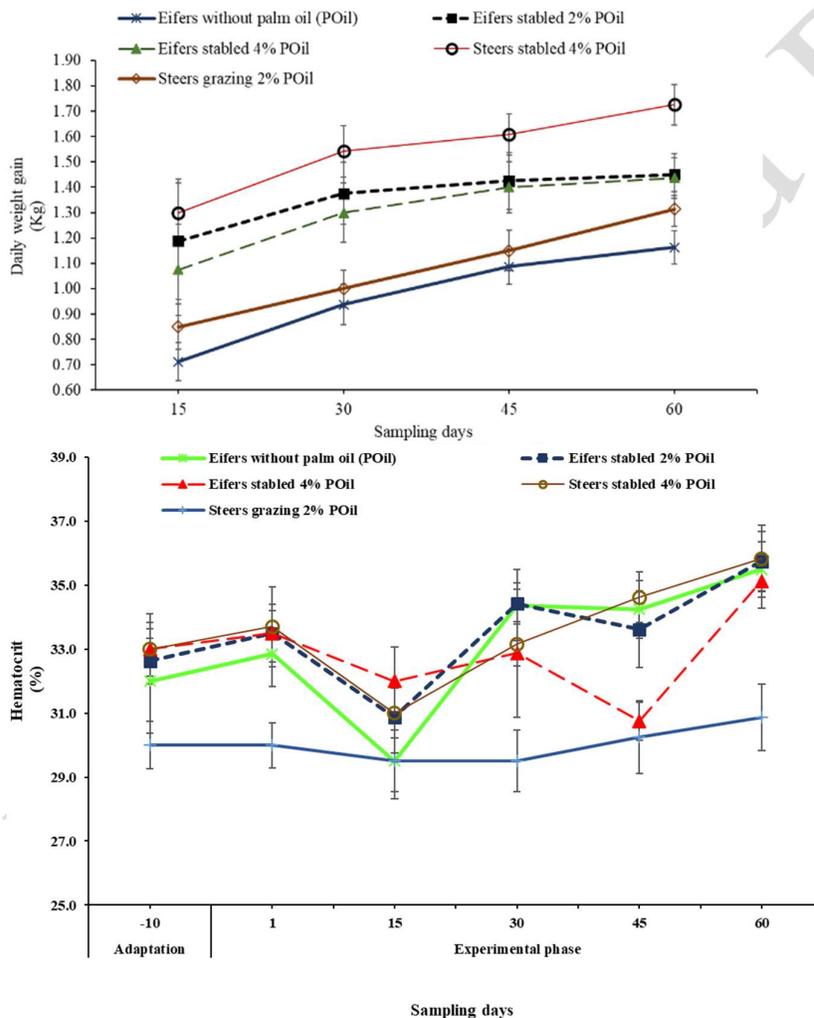


Fig. 1: Daily weight gains in heifers and steers supplemented with different proportions of palm oil.

Fig. 2: Hematocrit values during the study period in heifers and steers supplemented with palm oil.

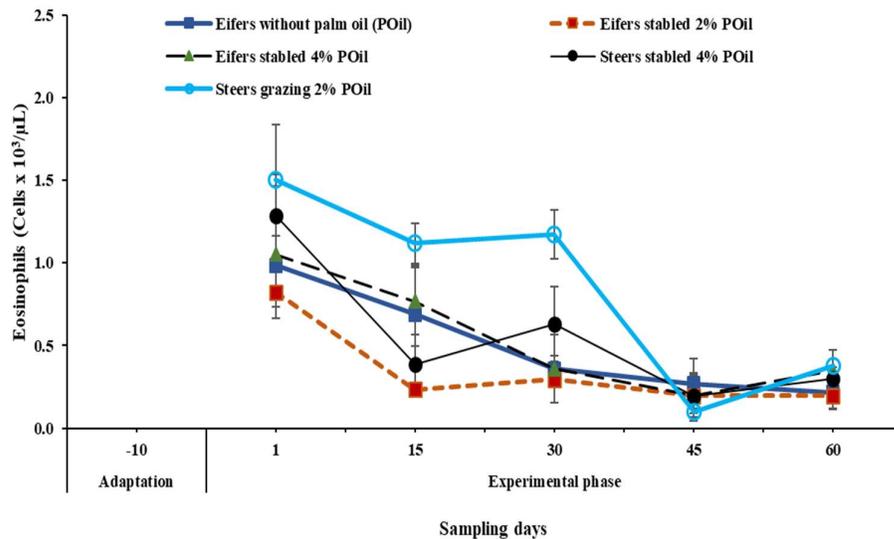


Fig. 3: Peripheral eosinophil count (mg/dL) in cattle supplemented with palm oil.

DISCUSSION

The integration of agricultural activities, such as POil production, and livestock production promotes an ecological relationship between different production systems, making the results of this study highly relevant. POil cultivation has spread widely in southeastern Mexico (Castellanos-Navarrete 2018), and the economic and environmental impact has been of great interest, as in other countries that produce this crop (Purnomo et al. 2020). The use of by-products from the palm industry in livestock represents a strategy to increase the supply of meat using resources in a sustainable manner (Ahmad and Nasir 2020). However, when fiber sources derived from palm are used to replace grass, ADG values similar to those recorded in grazing cattle under warm conditions have been obtained (Gomez-Vázquez et al. 2017). In other studies where African palm fiber was used to feed cattle, higher gains have been reported compared to grazing, but in general, the diets do not allow reaching more than 900g per day (Pimpa et al. 2021).

In the present study, ADG was significantly improved for all experimental treatments when POil was used as the main energy source, both in females and males. These values were higher than those obtained in the studies where African palm fibers were used, mainly because the oil is a concentrated source of energy (Martínez Marín et al. 2010). Various studies have reported that lipid supplementation increases the body weight of cattle, in addition to improving the color and thickness of dorsal fat as well as fat deposition in the muscle; however, the cost of feeding increases and reduces the net profit for the producer (Pimpa et al. 2021). The use of by-products of cassava and crude POil in native Brahman x Thai calves resulted in a reduced ADG of only 0.5kg per day (Phoemchalard et al. 2014). The lower ADG compared to that reported in the present study may be associated with the age and weight of the animals since that study used young animals (under 1 year of age) and with a body weight of 130kg, whereas those used in our study had an initial weight of 249kg. The differences in ADG observed between females and males is an aspect that generally occurs in cattle (Bureš and Bartoň

2012; Pogorzelska-Przybyłek et al. 2021). Males exhibit a greater ability to convert feed into muscle tissue, whereas females reach maturity earlier, resulting in better finished carcasses (Bonilha et al. 2015; Pogorzelska-Przybyłek et al. 2021).

The hematological parameters were similar between treatments, but a great difference was observed with the values reported in other studies. These differences can be attributed to multiple factors such as differences between races (González-Garduño et al. 2017), feeding and age of the animals (Botezatu et al. 2014), as well as climatic factors and the presence of diseases or parasites (Calzada et al. 2002). The physiological status is another factor that affects hematologic values. Thus, in this study, the number of RBC differs from the values reported by other authors. Lower values of RBC and HGB ($6-7 \times 10^3$ mL and 10.0 to 10.7g/dL) were found in the study carried out by Bedenicki et al. (2014), who sampled lactating cows of the Croatian Istria autochthonous breed. Similar results have been reported in Queensland, Australia, in Holstein-Friesian cows before and after calving, and the values were lower for RBC and HGB (Jonsson et al. 2013). However, Bedenicki et al. (2014) reported a high HCT in the Croatian breed. In general, beef cattle breeds have higher RBC values than dairy cattle. Roland et al. (2014) show that male cattle have higher HCT than cows and report higher RBC values in non-lactating than lactating cows. However, in the present study, the RBC was similar between females and males, because they were animals of the same age and with inactive reproductive stage.

In another study comparing different breeds, *Bos taurus* cattle had lower values in the erythron than *Bos indicus* and *Bos jabanicus* (Sofyan et al. 2020), which coincides with the values registered in the present work, in which the values in crossbred cattle of *B. taurus* x *B. indicus* supplemented with POil showed high values compared to those indicated in other breeds. In Angus cattle, a lower RBC count was observed, but a high content of HGB and HCT (Herd et al. 2019). In the present study, there were no differences due to the sex of the animals, although some other authors show a difference in RBC and HGB between females and males (Bonilha et al. 2015). Notable differences occurred only

between grazing cattle supplemented with POil. The values of RBC and HCT were similar to those indicated in Sahiwal cattle in a study that evaluated environmental factors during different seasons of the year (Chandra Bhan, Singh et al. 2012). Similarly, in another study conducted in Baja California, Mexico, high hematological profiles were shown in both European-type (Charolais and Simbrah) and Zebu-type heifers, with racial differences and by the type of shade used in their development in feedlots (Aguilar-Quiñonez et al. 2021).

Few investigations have reported the hematological changes that occur during the fattening phase of steers and heifers, making it important to show that there is a clear increase in the erythron after a 45-day stable period. All animals supplemented with a diet including POil showed an increase in HCT, RBC, and HGB. Although grazing calves took longer to increase their hematological profile at 45 days, their erythron was recovered without difference with the stabled groups. Parameters such as the RBC count could be related to physiological functions, including oxygen consumption and transport, and may differ due to changes in the metabolic rate. Additionally, white blood cell subpopulations may be associated with differences in immune function, which may also influence energy distribution. Positive relationships between feed efficiency and blood cell measures, including MCV, MCH, and lymphocyte count, have been observed in steers. These associations have also been shown in other species, with higher concentrations of red and white blood cells and a reduced mean corpuscular volume in sheep without efficient feeding (Cònsolo et al. 2018).

In the size of the red blood cells, no differences were observed among the different groups, and in comparison with other results, similar values are reported for young animals from 12 to 18 months of age (Botezatu et al. 2014). Cell size is directly related to age, and thus, younger animals have smaller red blood cells than larger animals (González-Garduño et al. 2019). On the other hand, before 8 weeks of age, the size is close to 40fL, with a reduction to 35fL until 25 weeks of age, followed by an increase. However, during in the first 6 months of the life of calves, the red cells are smaller than those in adult cattle. Low MCV values have been attributed to the replacement of RBC containing fetal hemoglobin with smaller RBC; however, the small size is compensated by the large number of RBC to maintain normal HGB values (Brun-Hansen et al. 2006). The MCV found in adult cattle from different studies coincides with an average of 46–47fL (Calzada et al. 2002). When observing the behavior over time, it was noted that MCV increased over time from a size of 38 to 43fL. Possibly, this increase in the size of the RBC is due to a regenerative response during the fattening process in the feed lot (Jones and Allison 2007). In another study in Gyr females, the MCV increased with age. In calves from one year of age, the size of the red blood cell is smaller (40fL) than in those of 2 years old (50fL) (Jacob et al. 2019).

White blood cell counts are directly related to the immune response of animals. Of the white blood cells, eosinophils are the most important cells and related to the protection and immune response against infections of gastrointestinal parasites (Jenvey et al. 2020; Mitre and Klion 2021). Eosinophilia is a typical consequence of

infection with helminths (Hendawy 2018). However, the response can vary drastically due to the effects of host factors such as genetic resistance and allergenic capacity; on the other hand, the characteristics of the parasite species are determinant in the eosinophilic response (Mitre and Klion 2021). In the present study, a reduction was observed after the start of the stable period. In grazing animals, the eosinophil count remained similar. However, at 45 days, the counts were reduced in all animals, most likely as a result of deworming, as eosinophilia tends to decrease over time in various chronic helminth infections (Mitre and Klion 2021).

Conclusion

The use of palm oil in cattle feeding in tropical regions represents a viable alternative to improve the energy balance of the ration, with a positive effect on the hematological parameters of the erythron, eosinophil count, and the health status of the cattle. In addition, it can improve the weight gain in stabled and grazing cattle, associated with better animal health.

Author Contributions: The experiment was conducted by EJ Flores and C Córdova. R González, G Sosa and A Villa contributed to the interpretation of the experimental results and the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding Source: This research was supported by the General Directorate of Research and Postgraduate Studies of the Universidad Autónoma Chapingo (21012-ECI-92).

Conflicts of Interest: The authors declare no conflict of interest.

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