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IRON LEVELS AND ERYTHROCYTE INDICES IN PIGLETS SUPPLEMENTED WITH CUSHURO (NOSTOC SPHAERICUM)

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ABSTRACT

The study investigates the impact of supplementation with dehydrated cushuro (Nostoc spherical) on iron levels and erythrocyte indices in lactating piglets. A total of 48 newborn Camborough piglets (24 males and 24 females) were used in the study, which was divided into three treatments: Group T1 was administered an intramuscular injection of dextran iron (200 mg). Groups T2 and T3 were orally supplemented with 50 or 100 g of dehydrated and ground cushuro per kg of their feed, respectively. The piglets had access to isoprotein and isocaloric feed and water in spaces adjacent to the mother's pen. Blood samples were collected for the purpose of determining erythrocyte indices and serum iron levels. Serum iron values were found to be comparable between groups at 7 days of age ($p > 0.05$) across different treatments and sexes. However, T2 values at 14 and 21 days of age were significantly lower than T1 and T3 ($p<0.05$). The erythrocyte values (red blood cell count [RBC]; hemoglobin [HGB]; hematocrit [HTC]; mean corpuscular volume [MCV]; mean corpuscular hemoglobin concentration [MCHC]) were found to be similar between treatments and sex at 7 days. However, at day 14, T1 piglets showed higher values of RBC, HGB and HTC with respect to T2 ($p < 0.05$). In addition, T2 at day 21 exhibited reduced erythrocyte indices in comparison to T1 and T3 ($p < 0.05$). It is evident that the provision of a ration comprising 10% dehydrated cushuro is sufficient to circumvent the manifestation of iron deficiency anemia in lactating piglets. This finding signifies that the ration can effectively substitute for the IM injection in intensively reared piglets, thereby ensuring the sufficiency of iron intake.

KEYWORDS: Piglets, Iron, Cushuro, Hemoglobin, Anemia.

1. INTRODUCTION

It is well-documented that piglets are susceptible to infectious and nutritional diseases at birth (Bellezze et al., 2011). Within the latter, anemia has been shown to be a cause of high morbidity (Pighin et al., 2016) and mortality (Góngora-Manzanero et al., 2004). The condition is characterized by a deficiency of iron, hence the nomenclature of iron deficiency anemia. It is estimated that piglets are born with 40 to 50 mg of iron as a reserve in the liver, sufficient to cover their requirements for the first three days of life (Quiles & Hevia, 2003). The intake of iron through breast milk is 1 mg/day (Quiles & Hevia, 2003), and it is recommended that piglets receive and retain between 7 and 15 mg of Fe per day (Quiles & Hevia, 2003; Jackson & Crockcroft, 2009). This type of anemia has been observed to manifest in two distinct forms. The first, known as the acute form, occurs between the second and third week of life. The second, the chronic form, manifests between weaning and the fifth week of age, particularly in large litters (Dybkjaer et al., 2006).

As demonstrated in the seminal study by Jackson and Cockcroft (2009), piglets are born with a hemoglobin concentration of 12 g per 100 ml of blood. By eight days of age, this concentration has decreased to 8 g per 100 ml (Jackson and Cockcroft, 2009). Similarly, the range of hematocrit is reported to be between 15 and 40%, a variation attributable to the influence of various factors on the absorption of Fe (Quiles and Hevia, 2003). Iron deficiency has been shown to produce microcytic and hypochromic anemia (Góngora-Manzanero et al., 2004) due to the reduced levels of hemoglobin and diminished size of the erythrocytes (Cansaya, 2017). Consequently, iron supplementation is imperative for the effective management of anemia in piglets.

It is standard practice for piglets to receive intramuscular injections of 200 mg of Fe dextran between the first 3 to 7 days of birth. However, the implementation of this application has been observed to induce stress in piglets, in addition to necessitating inputs and trained personnel for its execution. Inadequate practices have been demonstrated to result in the formation of abscesses (Shimada, 2007) and infections caused by Clostridium spp., which can lead to toxemia and death within 12 to 24 hours following injection (Andresen, 1990). As Lagos (2015) also highlighted, there is a risk of acute toxicity due to excess iron. Pérez et al. (2005) posits that both deficiency and excess of this essential metal are deleterious.

Nostoc sphaericum, known by various names including cushuro, murmunta and llullucha, possesses the potential to become an excellent functional food, especially in combating deficiency diseases such as anemia in humans (Alvarado & Rodríguez, 2017). The pig's gastrointestinal tract has been shown to exhibit a high degree of similarity to the human one in terms of digestive processes and iron absorption. This suggests the potential for cushuro to combat iron deficiency anemia in piglets. In consideration of the points, the objective of the present study was twofold: firstly, to evaluate the effect of cushuro supplementation in concentrated feed, and secondly, to measure iron levels and erythrocyte indices in lactating piglets until early weaning.

2. MATERIALS AND METHODS

The research was conducted at the piglet demonstration module of the IVITA Huaral Station of the Faculty of Veterinary Medicine (FMV), Universidad Nacional Mayor de San Marcos (UNMSM), which is in the district of Huaral, department of Lima, Peru.

A quantity of 160 kg of fresh cushuro was used in the process, which was then subjected to a drying procedure at a temperature of 60°C for a duration of 72 hours, employing the use of a stove. After the drying process, the material was subjected to a milling procedure. Depending on the specific treatment applied, the milled material was then added to the feed, in accordance with the required dosage. The proximal analysis of the dehydrated cushuro was conducted at the Laboratory of Biochemistry, Nutrition and Animal Feed (LBNAA) of the FMV-UNMSM, while the iron content analysis was carried out at the Institute of Nutritional Research in Lima, employing the AOAC 975.03 methodology.

Table 1: Proximal analysis of cushuro (Nostoc sphaericum).

	Values
Humidity (%)	16.1
Crude protein (%)	43.6
Fats (%)	1.21
Crude fibre (%)	6.1
Ashes (%)	4.1
Iron (ppm)	803.6

Source: Prepared based on analyses carried out at the Anima Biochemistry, Nutrition and Food Laboratory (FMV-UNMSM) and the Nutritional Research Institute

The experiment used a total of 48 newborn piglets (24 males and 24 females) from four litters of mothers of the Camborough meat line. Lactating piglets were

identified and randomly distributed into three treatments. The piglets were separated from their mothers during the day on three occasions in spaces adjacent to the pen for a period of one hour. During this time, the piglets received water and concentrated feed according to their treatment group (Figure 1). This process was conducted from day 3 to day 21 of age. During the course of the research, the microclimate was maintained within the range of 30 to 32°C, with an average ambient humidity of 88%.

The experimental design encompassed three distinct treatments: T1: The experiment comprised two groups of piglets: the first group was fed a base diet, while the second group was given an intramuscular injection of dextran iron (200 mg) on the third day of birth. The experiment was conducted on days 3 and 4 (T2 and T3, respectively). The subjects were fed a base diet and 5 or 10% dehydrated cushuro per kilogram of food, respectively, from day 3 of birth (Figure 1).

Figure 1: Concentrated feed offered to piglets with the addition of cushuro (Nostoc sphaericum).

Table 2: Nutritional composition of the ration of T1 treatments with Dextran Iron or with cushuro supplementation (Nostoc sphaericum) T2 5% and T3 10%.

Inputs	Percentage (%)		
	T1	S2	S3
Corn grain - 8.80% gb	59.2	56	53
Soy cake - 46% BW	29.8	28.5	27
Plasma	3.3	3	3
Dehydrated cushuro in flour	0	5	10
Soybean Oil	3	2.9	2.6
Digest fast	0.1	0.1	0.1
L-lysine	0.3	0.3	0.3
Dl-methionine	0.1	0.1	0.1
L-threonine	0.1	0.1	0.1
L-tryptophan	0.1	0.1	0.1
Calcium carbonate	1.1	1.1	1
Dicalcium phosphate	1.9	1.8	1.7
Salt	0.5	0.5	0.5
Premix vitamins and minerals	0.1	0.1	0.1
Fungal inhibitor	0.1	0.1	0.1
Mycotoxin sequestrant	0.2	0.2	0.2
Copper Sulfate	0.05	0.05	0.05
Zinc oxide	0.05	0.05	0.05
Total	100.00	100.00	100.00

For hematological analysis and serum iron levels, two blood samples (2 ml) were taken from the anterior vena cava of each piglet on days 7, 14 and 21 of age in Vacutainer tubes with EDTA-K3 anticoagulant for the complete blood count and in tubes with coagulation activator (activating clot) to determine serum iron values. To this end, the piglets were inhaled with isoflurane according to the following protocol: Induction through the use of a mask: 3% isoflurane was administered in 100% oxygen to make the animal lose consciousness and then reduced to 1.5% isoflurane in 100% oxygen for maintenance with an oxygen flow of 2 l/m., which allowed us to take the samples in just two minutes at most. Complying with the animal welfare protocol approved by the Ethics and Animal Welfare Committee of the FMV-UNMSM (CEBA, 2021).

The blood samples with anticoagulant were taken to the Rayto hematological analyzer model RT-7600 Veterinarian and to determine ferritin in the serum the Riele spectrophotometer model 2010 V5+ and the Iron FS reagent from the DIASYS laboratory (Germany) were used. For statistics, analysis of variance (ANOVA) and Duncan's test were used through the statistical package IBM SPSS, 2022) with a significance level of 0.05.

3. RESULTS

Table 3: Erythrocyte indices 1 and iron indices in 7-day-old piglets with intramuscular administration of Fe Dextran or with cushuro supplementation (Nostoc sphaericum) by sex.

		T1 Dextran Iron	S2 Cushuro 5%	S3 Cushuro 10%	Rank
Hematological analysis	RBC, x106/uL	4.426 ± 0.291a	4.584 ± 0.147a	4,538 ± 0.255	4.00 – 4.99
	Males	4,416th	4,536th	4,458th	
	Females	4,436th	4,633rd	4,618th	
	HGB, g/dL	9.570 ± 0.72a	9.16 ± 0.55a	9.11 ± 0.72a	7.90 – 10.80
	Males	9.59th	9.14th	8.96th	
	Females	9.55th	9.18th	9.25th	
	HTC, %	25.74 ± 1.59th	26.18 ± 2.53rd	25.45 ± 3.49th	20.50 – 31.60
	Males	26.12th	26.55th	25.19th	
	Females	25.35th	25.80th	25.71st	
	MCV, fL	58.40 ± 5.42nd	57.09 ± 5.10th	56.14 ± 7.40th	43.30 – 70.60
	Males	59.53rd	58.46th	56.67th	
	Females	57.27th	55.72nd	55.62nd	
	MCHC, g/dL	37.20 ± 1.26th	35.31 ± 4.13th	36.12 ± 3.14th	28.60 – 44.50
	Males	36.70th	34.66th	35.97th	
	Females	37.69th	35.96th	36.26th	
Serum Iron	Fe Serum, µg/dL	144.56 ± 32.85th	142.25 ± 17.05th	140.62 ± 28.00a	105 - 199
	Males	145.50th	141.13a	142.50th	
	Females	143.63rd	143.38th	138.75th	

[1] RBC: Red blood cell count; HGB: Hemoglobin; HTC: Hematocrit; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration

Table 4: Erythrocyte indices1 and iron indices in 14-day-old piglets with intramuscular administration of Fe Dextran or with supplementation of cushuro (Nostoc sphaericum) according to sex.

		T1 Dextran Faith	S2 Cushuro 5%	S3 Cushuro 10%	Rank
Hematological analysis	RBC, x106/uL	5.474 ± 0.501a	4.803 ± 0.346b	5.256 ± 0.677a	5.007 – 5.348
	Males	5,364th	4.694b	4.874ab	
	Females	5,584th	4.913b	5,639th	
	HGB, g/dL	11.26 ± 1.15th	8.42 ± 1.71b	10.34 ± 1.58b	9.457 – 10.556
	Males	11.25th	8.50b	9.58b	
	Females	11.26th	8.34b	11.11th	
	HTC, %	33.68 ± 4.92nd	28.04 ± 2.88 [b]	30.81 ± 5.64ab	29.36 – 32.32
	Males	33.72nd	27.20b	27.61b	
	Females	33.64th	28.89b	34.00A	
	MCV, fL	61.71 ± 8.77th	58.78 ± 8.27th	58.51 ± 7.87th	57.27 – 62.07
	Males	63.20th	58.40th	56.37th	
	Females	60.22nd	59.16th	60.65th	
	MCHC, g/dL	33.76 ± 3.21st	30.50 ± 7.63rd	33.90 ± 3.04th	31.20 – 34.24
	Males	33.52nd	31.80th	34.88th	
	Females	34.00th	29.20th	32.92nd	
Steel rod	Fe serum, µg/dL	306.56 ± 5.70th	186.31 ± 6.83b	269.44 ± 67.10th	134 – 457
	Males	323.75th	186.38b	265.13a	
	Females	289.38a	186.25b	268.75th	

[1] RBC: Red blood cell count; HGB: Hemoglobin; HTC: Hematocrit; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration

Table 5: Erythrocyte indices1 and iron indices in 21-day-old piglets with intramuscular administration of Fe Dextran or with cushuro supplementation (Nostoc sphaericum) by sex.

		T1 Dextran Faith	S2 Cushuro 5%	S3 Cushuro 10%	Rank
Hematological analysis	RBC, x106/uL	5.488 ± 0.571a	4.911 ± 0.474 [b]	5.412 ± 0.510a	4.21 – 6.60
	Males	5.54th	4.81b	5,385th	
	Females	5.44th	5.01b	5,439th	
	HGB, g/dL	11.10 ± 1.17th	9.01 ± 1.26b	10.89 ± 12th	6.30 – 12.60
	Males	11.61st	8.72b	11.34th	
	Females	10.59th	9.29b	10.44th	
	HTC, %	32.77 ± 4.16th	28.74 ± 3.05b	33.14 ± 4.31a	22.20 – 39.40
	Males	34.02nd	28.24b	33.84th	
	Females	31.51[a]b	29.25b	32.45[a]b	
	MCV, fL	61.97 ± 7.85th	53.8 ± 5.50b	62.41 ± 5.81st	41.60 – 76.80
	Males	62.28th	54.16b	62.78th	
	Females	61.66th	53.47b	62.04th	
	MCHC, g/dL	33,736 ± 3.94th	29.970 ± 3.83 [b]	33,662 ± 2.63rd	19.90 – 43.20
	Males	34.18th	28.61b	34.38th	
	Females	33.29th	29.33b	32.94ab	
Steel rod	Fe serum, µg/dL	210.94 ± 39.22nd	153.63 ± 26.63 [B]	210.81 ± 44.86th	108 – 299
	Males	199.00a	141.50b	209.38th	
	Females	222.88a	165.75b	212.25a	

[1] RBC: Red blood cell count; HGB: Hemoglobin; HTC: Hematocrit; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration

The control group, which received an injection of dextran iron at three days of age, exhibited a surge in blood Fe levels during the second week. This was followed by a decline in the third week, resulting in levels that were 10% of the treatment level with cushuro. Conversely, the latter exhibited an escalating level of Fe during the three-week period of the study.

4. DISCUSSION

On the seventh day of the experiment, the erythrocyte indices and serum iron levels of the three experimental groups were comparable. This is because piglets are capable of meeting their iron requirements through their liver reserves and the modest amount they receive from their mother's milk (Andresen, 1990). The range of hemoglobin values in piglets from 3 to 21 days of age has been documented as from 8.6 ± 1.3 to 11.3 ± 1.1 g/dL (Leman et al., 1986; Dybkjaer et al., 2006). These values are analogous to those observed in the present study.

In the second week of life, significant alterations in the erythrocyte indices of piglets with minimal Fe intake in the feed were observed. Consequently, individuals of T2 (cushuro 5%), both males and females, exhibited reduced levels of hemoglobin and hematocrit. In a similar manner, T3 males (cushuro 10%) exhibited a reduced level of hemoglobin, potentially attributable to their accelerated growth rate. During this process, iron is initially utilized to synthesize myoglobin and enzymes, followed by its incorporation into hemoglobin (Cansaya, 2017).

During the third week of the experiment, signs of iron deficiency became apparent, as evidenced by lower erythrocyte values in T2. The values obtained could be indicative of acute iron deficiency anemia, given its presentation with hypochromic characteristics due to low mean corpuscular hemoglobin (MCHC) values and microcytic characteristics related to low mean corpuscular volume (MCV) values. These outcomes are attributable to a deficiency in hemoglobin synthesis due to Fe deficiency (Day et al., 2012). Although anemia is generally microcytic and hypochromic, there appears to be a decrease in the number of erythrocytes (Cansaya, 2017). However, it is imperative to exercise caution when interpreting red blood cell count results, as the number of red blood cells varies significantly between species and within individuals of the same species (Dukes, 2004). The following text is intended to provide a comprehensive overview of the subject matter.

Zevallos (2008) discovered that piglets administered oral dextran iron on the first day of birth exhibited higher hematocrit levels at seven days post-treatment (31.6%), while those that received intramuscular dextran iron on the second day of birth demonstrated a decline in hematocrit (30.6%). The hematocrit levels in the intramuscular dextran iron group decreased further by the 14-day mark, reaching 27.5%, while the oral dextran iron group exhibited a 30.0% hematocrit level. These values were lower than those found in the present study when Fe was administered orally and injectable.

The findings of this study suggest that the oral administration of Fe to lactating piglets results in effects that are analogous to those of the injectable formulation. Conversely, Andresen (1990) posited that this method engenders a reduced level of stress in the animal. Conversely, it is imperative to habituate the piglets to the early consumption of feed. This is to ensure that when the milk supply of the piglet diminishes, the deficit can be addressed with the pre-start ration (Shimada, 2007).

5. CONCLUSIONS

The study concludes that supplementing lactating piglets with a ration containing 10% dehydrated cushuro is effective in preventing iron deficiency anemia. This finding suggests that the 10% cushuro ration can be a viable substitute for the standard intramuscular injection of dextran iron in intensively reared piglets. By replacing the injection, this dietary supplementation avoids the stress associated with the procedure while still ensuring adequate iron intake and preventing anemia.

Compliance with Ethical Standards

The study was carried out in accordance with the guidelines of the Declaration of Helsinki for the compliance with the ethical, methodological, and legal aspects of this study, as well as the informed consent for the processing of personal data.

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The research was conducted independently by the researchers, and no funding was received.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Contribution: All authors contributed equally to this work

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Abbreviations

AOAC: Association of Official Analytical Chemists
CEBA: Ethics and Animal Welfare Committee of the FMV-UNMSM
Fe: Iron
FMV: Faculty of Veterinary Medicine
HGB: Hemoglobin
HTC: Hematocrit
IM: Intramuscular
LBNAA: Laboratory of Biochemistry, Nutrition and Animal Feed
MCHC: Mean Corpuscular Hemoglobin Concentration
MCV: Mean Corpuscular Volume
RBC: Red blood cell count
UNMSM: Universidad Nacional Mayor de San Marcos

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