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Iron interference in hemoglobin production in piglets from birth to weaning[1]

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ABSTRACT.- Cruz N.R.N., Baraldi T.G., Marinho Neto F.A., Alvarenga P.V.A., Oliveira J.P., Albuquerque A.C.A, Brito H.C.D., Nascimento L.A.N, Oliveira L.G. & Santana A.E. 2023. **Iron interference in hemoglobin production in piglets from birth to weaning**. *Pesquisa Veterinária Brasileira 43:e07161, 2023*. Departamento de Clínica e Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), Via Acesso Prof. Doutor Paulo Donato Castelanni Km 6, Jaboticabal, SP 14884-900, Brazil. E-mail: nathancruzbr@gmail.com

Iron deficiency anemia (IDA) in humans is defined as the decrease of total hemoglobin concentration and the non-production of the adult hemoglobin subtype 2 – HbA2 (α2δ2 chains), which is considered a marker of IDA severity in humans, dosed together with the iron serum. This study aimed to determine the standard of hemoglobin types in piglets induced to experimentally IDA in the first 21 days of life (delivery to weaning). In the present study, 40 piglets born from four naïve gilts, were randomly and equally assigned among the gilts. On the third day after delivery, the groups were randomly distributed in different environments (cement and clay floors) and according to the iron supplementation (iron dextran and placebo). Erythrocyte parameters, serum iron, and hemoglobin trait were analyzed at four moments between birth and weaning days. The group of piglets that did not receive iron dextran supplementation on the third-day post-birth and were placed in the pen without soil did not present HbA2 from the seventh day onwards on the agarose electrophoretogram (pH 8.6) and this observation was correlated to decrease of serum iron (ρ: 0.156, *p*=0.003) when compared to the other groups' piglets that did not present iron deficiency. In the present study was possible to determine the swine hemoglobin pattern in IDA, since HbA2 was absent in piglets with IDA in comparison to the non-ferropenic groups and the correlation between the reduction of iron levels and the absence of HbA2.

INDEX TERMS: Swine, hemoglobinopathy, iron deficiency anemia, electrophoresis, HbA2, piglets, pigs.

RESUMO.- [**Interferência do ferro na produção de hemoglobina de leitões do nascimento a desmama.**] A anemia por deficiência de ferro (ADF) em humanos é definida como a diminuição da concentração de hemoglobina total e a não produção da hemoglobina adulta subtipo 2 – HbA2 (cadeias α2δ2), que é considerada um marcador de gravidade de IDA em humanos, dosado em conjunto com o soro de ferro. Este estudo teve como objetivo determinar o padrão dos tipos de hemoglobina em leitões induzidos experimentalmente à IDA nos primeiros 21 dias de vida (parto ao desmame). Quarenta leitões, nascidos de quatro marrãs nulíparas, foram distribuídos aleatoriamente e igualmente entre as leitoas. No terceiro dia após o parto, os grupos foram distribuídos aleatoriamente em diferentes ambientes (piso de cimento e barro) e de acordo com a suplementação de ferro (ferro dextrano e placebo). Parâmetros eritrocitários, ferro sérico e traço de hemoglobina foram analisados em quatro momentos, entre o nascimento e o desmame. O grupo de leitões que não recebeu suplementação de ferro dextrano no terceiro dia pós-parto e foi colocado em baia sem solo não apresentou HbA2 a partir do sétimo dia no eletroforetograma de agarose (pH

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8,6) e esta observação foi correlacionada com diminuição da concentração sérica ferro (ρ: 0,156, *p*=0,003) quando comparados aos demais grupos leitões que não apresentavam deficiência de ferro. No presente estudo foi possível determinar o padrão hemoglobinêmico suíno na IDA, uma vez que, a HbA2 estava ausente nos leitões com ADF em comparação aos grupos não ferropênicos e há correlação entre a redução dos níveis de ferro e a ausência de HbA2.

TERMOS DE INDEXAÇÃO: Suínos, hemoglobinopatia, anemia ferropriva, eletroforese, HbA2, leitões, porcos.

INTRODUCTION

Hemoglobin (Hb) is a globular protein composed of a protein fraction (globin chains) and a prosthetic fraction represented by protoporphyrin-9, in which the divalent ion of iron (Fe ++) binds (Harvey 2012). After birth, mammals have three types of hemoglobins: adult hemoglobin – HbA (α2β2 chains) majority fraction of total hemoglobin (>95%); fetal hemoglobin – HbF (α2γ2 chains) (2.5-4.0%); and adult hemoglobin 2 – HbA2 (α2δ2 chains) 1% of the total hemoglobin concentration (Bain 2006). After the last third of gestation of the mammals (cats, dogs, horses, and pigs) HbF is gradually replaced by HbA and HbA2 (Wild & Bain 2007).

When iron deficiency occurs, the hemoglobin molecule may suffer from oxidation, especially in the heme fraction. This mineral is inserted, resulting in hemoglobin subtypes decreasing or interrupting its production (El-Agouza et al. 2002). In the Middle East, human beta-thalassemia is a usual type of hemoglobinopathy, characterized by β-chain changes and increases in HbA2 production (Denic et al. 2013); diagnosed by hemoglobin electrophotography. However, if the beta-thalassemic individual develops iron deficiency it will decrease the production of HbA2 (Mosca et al. 2008). Thus, this fact can make it difficult to diagnose the beta-thalassemia condition due to the lack of production of this type of hemoglobin (Giambona et al. 2009).

IDA causes a mortality rate of up to 80% in piglets raised in a cement environment and did not receive iron dextran as breast milk supplementation (Santana 1982, Mores et al. 1998). This significant rate is due to the rapid weight gain of litter, low iron body stores, high iron requirement from the second to the third week of life, and low breast milk levels of iron (Bhattarai & Nielsen 2015), which result in the spoliation of body ferric stocks, anemia and immunological depletion (Nunes et al. 1997).

IDA in humans is also the most prevalent cause of deficiency diseases in children from 0 to 5 years old and pregnant and is related to a decrease in cerebral, cognitive, immunological, physical, and productive development (WHO 2004, De Benoist et al. 2009, Antonides et al. 2015).

The iron demand in a human occurs from six months of life with a peak at 12 months, requiring an iron daily accumulation of 8%, used for erythropoietic differentiation, muscle formation, and replacement of fetal (HbF) to adult hemoglobin types (HbA and HbA2) (Ozdemir et al. 2013). In piglets, it happens in the first weeks of life (7 to 14 days of age) (Carvalho et al. 2006, Rytych et al. 2012).

The studies about hemoglobin behavior and pathology in animals are not frequent when compared to human hemoglobin studies. Some authors reported the occurrence of hemoglobinopathies in animals such as beta-chain acetylation in cats (Taketa et al. 1972), methemoglobin formation in cats with toxemic conditions (Fighera et al. 2002), glycosylation of Hb in diabetic dogs due to glucose (Elliott et al. 1997) and carbamylation by a prolonged increase of serum urea in dogs with acute renal disease (Heiene et al. 2001). Despite iron deficiency in newborn and suckling piglets being a well-established and consolidated topic in swine science, little is known about the hemoglobin ratio and behavior, as well as the relationship with IDA are poorly studied.

Therefore, the present study aimed to determine the standard of hemoglobin types in piglets submitted to experimentally induced IDA in the first 21 days of life (birth to weaning).

MATERIALS AND METHODS

Research location local and contextualization. In the present study, 40 piglets born of four naïve gilts (Naïma herd, Choice Genetics, São Paulo, Brazil), with ages from 150 to 165 days, weight from 90 to 100kg, vermifuge (Ivermectina, dose: 100mcg/kg/day during seven days on feed); inseminated; vaccinated against erysipela, parvovirus, and leptospirosis were included (FarrowSure® B Gold, Zoetis). Permission to conduct the study was received by the Ethics Committee on the Use of Animals (Protocol #05824/14).

Experimental design. On the delivery day (Day 0), the piglets after receiving the colostrum were randomized and divided equally among the gilts (10 piglets/gilts), in a total of four experimental groups individually allocated into four pens in the same room. On the third day after delivery (D3), the groups were randomly divided according to the experimental model described by Santana (1982) and reproduced by Bhattarai & Nielsen (2015) regarding the environment (type of pen floor) and iron supplementation.

Regarding the environment, two groups were allocated in cement floor pens and two in clay floor pens. Two groups received iron supplementation (200mg of Iron Dextran, intramuscular injection, Ferrodex, Fabiane Saúde Animal, São Paulo, Brazil) and two groups received the placebo injection (sterile injectable saline 0.85% per intramuscularly) (Table 1).

After establishing the experimental conditions, the groups received the identification (Table 1): G1 = cement floor with iron supplementation; G2 = clay floor with iron supplementation; G3 = clay floor with the application of saline solution; G4 = cement floor with the application of the saline solution.

Samples collection. All piglets from the four experimental groups were collected for blood samples on day 3 (D3 – before the determination of the experimental condition), Day 7 (D7), Day 14 (D14), and Day 21 (D21), through jugular vein puncture, with hypodermic needles and syringes in EDTA-K2 anticoagulant tubes (for erythrocyte analysis and hemoglobin electrophoresis) and without anticoagulant (determination of serum iron).

Table 1. Experimental conditions of four groups of piglets according to the treatment used

Group	Pen floor	Iron supplementation
G1	Cement	2mL or 200mg iron dextran/piglets/single dose/IM
G2	Clay	
G3	Clay	2mL de sterile saline 0.85%/single dose/IM
G4	Cement	

IM = intramuscular injection.

Hematological and iron analysis. To perform the erythrogram, the automatic blood cell counters ABC Vet-ICHOR (ABX Horiba, Montpellier, France), pre-adjusted for the swine species, were used and the erythrocyte reference values reported by Thorn (2008) in the comparison of the results of this study for hemoglobin, hematocrit, red blood cell count (RBC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

A commercial kit (Serum Iron, Labtest Diagnóstica, Minas Gerais) was used for the determination of serum iron levels using the Goodwin colorimetric method and spectrophotometric determination in semi-automatic apparatus (Labquest Bioplus, Labtest Diagnóstica, Minas Gerais).

Hemoglobin electrophoresis. For the study of hemoglobin, types were performed the electrophoretic technique in agarose gel described by Lepp & Bluestein (1978) and preparation of the sample with chloroform hemolysis for electrophoretic analysis, proposed by Bonini-Domingos (2003). A portion of blood with anticoagulant was centrifuged FCR of 800G for 5 minutes. The plasma fraction was discarded, and the erythrocytes were washed three times with 0.85% saline. After washing, the supernatant was discarded, and the volume of washed erythrocytes was added to an equal volume of distilled water. Thereafter, an equal volume of chloroform P.A was added, and the solution was vigorously stirred and centrifuged again at 900G for 15 minutes. In the end, the supernatant solution was composed of free hemoglobin.

Agarose electrophoresis used buffer solution for the gel and Tris-Edta-Borato (TEB) tris at pH 8.6 (10.2g Tris hydroxymethyl aminomethane, 0.6 g, ethylenediaminetetraacetic acid, 3.2g boric acid and water distilled qs 1000mL). The 1% cementing agarose was equipped for 1 minute in microwaves for polymerization and was then in Fisher (FisherBiotech, FB-SB-1316, Fisher Scientific, Pittsburgh/PA). After gel solidification, the sample wells were formed, and the gel carrier was positioned for the negative pole (cathode).

The running buffer and the hemoglobin solution samples were added to the wells of the gel, the run was performed at 100 volts for 70 minutes or until good visualization of the electrophoretic separation. At the end of the run, the gel was stained with Coomassie blue (2.0g Coomassie blue, 50mL glacial acetic acid and 950mL distilled water) (Lepp & Bluestein 1978). The human hemolysate standard for sickle cell disease, which presents traces in HbS and HbA, was used as the sample, and the results were compared with the hemoglobin C, S, F, and A facsimile described by Lepp & Bluestein (1978) and Bain (2006) and expressed qualitatively (Fig.1).

Statistical analysis of the data. The statistical design was characterized by a completely randomized block in a 2 x 2 factorial scheme, considering two environments (cement and soil) versus iron supplementation (supplemented or not supplemented) and having an experimental plot and repetition of the adopted treatment of the piglets (environment vs. treatment). All analyzes were conducted in the Statistical Analysis System (SAS v.9.3, Cary, North Carolina) software. The results were described in mean and standard deviation after the Shapiro-Wilk normality test, submitted to the F statistic and the analysis of variance using the Tukey statistical model ($p \leq 0.05$), considering the significant difference between the groups in repeated measures, having a longitudinal factor (time) and the VC matrix for the interaction (treatment vs. time).

The Spearman correlation test ($p \leq 0.05$) was used to evaluate the correlation of the frequency of hemoglobin types with serum iron levels. G2 and G3 groups lost two piglets by smashing, the authors decided to not add or transfer piglets to these groups, to keep the experimental randomness condition concerning piglets, since this cause of mortality is common in the management of litters and the event did not compromise the degrees of experimental freedom. Thus, 38 piglets participated as the experimental number of this study.

RESULTS

The results of the hemoglobin types, serum iron concentration, and erythrocyte results are available in Table 2. The photo documentation of electrophoretic hemoglobin runs, and the form of interpretation compared to human standard positions (Fig.1) and hemoglobin trait proposed by Bonini-Domingos (2003).

The running on electrophoresis of piglets' hemoglobin presented similar to human hemoglobin standard (HbA + HbS) making it possible to verify that the HbA of the piglets runs in the same band as human HbA (Fig.1).

On D3, there was no statistically significant difference between the studied groups regarding hematological results (Table 2). At this point, all the groups presented reduced values in hematocrit values, red blood cells, and hemoglobin compared to the reference values (Thorn 2008). The groups G1, G2, and G3 presented the same hemoglobin subtype pattern (HbA + HbF) on electrophoresis, and the G4 group presented, besides HbA and HbF, the presence of HbA2.

On D7, G1 and G2 groups presented HbA2 differing from the G4 group, which did not present this subtype in comparison to the previous moment. Regarding the group presenting IDA (G4) in the same period was possible to note a similar line as HbS of the human standard that runs above HbA2 (Fig.1), resulting in HbA + HbF + HbS pattern in this group. The hemoglobin patterns of the four experimental groups were presented in Table 2. Group G4 presented anemia (Hb: 5.6±0.9g/dL), classified as microcytic (VCM: 48.9±1.3g/dL) and normochromic (MCHC: 30.7±1.2g/dL), with a significant decrease in the hemoglobin value when compared to the other experimental groups at the same time and lower than the presented values at D3 (Hb: 7.9±0.7g/dL). Besides the serum iron level in the same group was lower (113±15µg/

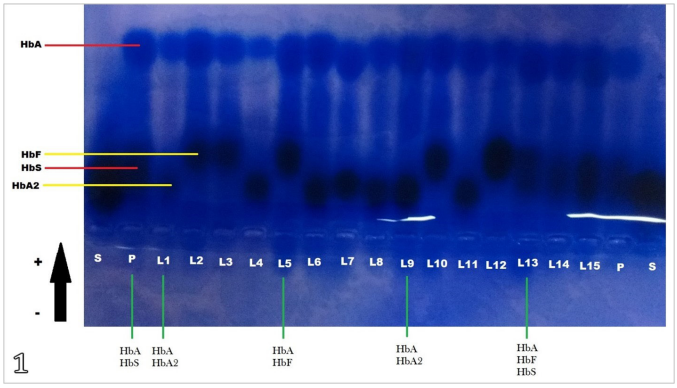

Fig.1. Piglet presenting hemoglobin trait in agarose gel electrophoresis pH 8.6. S = bovine albumin purified – electrophoresis control. P = human standard HbA/HbS (sickle cell trait). Lanes of electrophoresis (L1 to L15) = piglet's samples. Red lines = adult hemoglobin (HbA) and sickle cell hemoglobin (HbS) of human standard. Yellow lines = fetal hemoglobin (HbF) and A2 hemoglobin (HbA2) in piglet samples. Green lines = interpretation of the electrophoretic profiles with the human standard.

dL) than the reference value (121±33µg/dL), thus, the piglets of this group presented IDA.

On the other hand, G1 and G2 groups (215 and 197µg/dL, respectively), which received iron dextran supplementation presented higher and statistically significant iron levels when compared to groups that did not receive the iron doses (G4 = 145µg/dL and G3 = 113µg/dL). However, G4 presented higher statistical significance compared to group δ in serum iron and erythrogram results, but similar results with other groups (G1 and G2) in hematocrit, red blood cell (RBC), hemoglobin, MCV, and MCHC.

Still, in D7, the erythrogram results confirmed the IDA in piglets that did not receive iron supplementation and were kept on cement floor pens, and this condition remained until the end of the experimental period (D21).

In D14, the groups without IDA (G1, G2, and G3) had a similar hemoglobin pattern (HbA + HbF + HbA2) and this observation might be related to iron supplementation, once the G4 group showed only HbA + HbS and absence of HbF and HbA2. At weaning time (D21), the groups which not presented IDA (G1, G2, and G3) had HbA + HbF, and HbA2 in those groups where iron dextran supplementation was made. The G4 group (IDA) showed a different hemoglobin pattern when compared to the other groups since only presented HbA.

The decrease in iron levels presented a positive correlation with the absence of HbA2 band migration in the electrophoresis (ρ: 0.156, p=0.003). At D3, only G4 piglets presented the trace for HbA2, and from D7 onwards, all groups presented the trace for HbA2 at some point of the study except the G4 group, which did not present the trace at an experimental moment.

DISCUSSION

The low erythrocyte values of all groups at the first experimental moment (D3) may reflect the blood loss at birth, adaptation of the hematopoietic system of piglets, and persistence of fetal hemoglobin during the first week of life (Harvey 2012).

The different behavior of the iron levels between the groups on D7 was similar when compared with the literature by Starzyński et al. (2013), Wang et al. (2014), and Bhattarai & Nielsen (2015). Our results can be explained by the previous literature founds, who compared piglets with and without iron supplementation, and the piglets without iron supplementation and breed in cement pens developed IDA from the seventh day after birth. Although group G3 did not receive iron dextran and with low serum iron values on D7, this piglets group had an iron inclusion in their diet through the clay ingestion compared to group G4.

At the moments D14 and D21, G1, G2, and G3 groups presented erythrocyte parameters within the reference's values, while piglets of the G4 group had even more marked anemia associated with iron deficiency (Santana 1982, Starzyński et al. 2013, Bhattarai & Nielsen 2015, Szudzik et al. 2020). Confirming that the iron deficiency quickly induces the establishment of anemia, as seen in group G4, because of iron demand on the synthesis of new red blood cells during the second week of life (D14), differently from humans, which happens around the sixth month of age (Ozdemir et al. 2013).

The high values of MCHC in the G4 group at D14 are justified by the increase of free hemoglobin in the bloodstream, once the low levels of iron induce the hemoglobin oxidation and the susceptibility of erythrocyte lysis, resulting in the

Table 2. Results of hemoglobin trait, serum iron, hematocrit, red blood cells (RBC), hemoglobin, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) of piglets at days 3, 7, 14, and 21 after delivery

Day	Groups*	n	Hemoglobin trait[I]	Serum iron (121±33µg/dL)[II]	Hematocrit (32-50%)[III]	RBC (5.0-8.0 x 10^6/µL)[III]	Hemoglobin (10-16g/dL)[III]	MCV (50-68fL)[III]	MCHC (30-34%)[III]
3	G1	10	HbA + HbF	137 ± 12 a	21.6 ± 2.9	3.5 ± 0.3	7.2 ± 0.7	61.3 ± 3.8	33.2 ± 1.2
	G2	9	HbA + HbF	142 ± 19 b	24.9 ± 3.2	3.9 ± 0.5	7.6 ± 1.1	64 ± 0.9	30.6 ± 0.9
	G3	9	HbA + HbF + HbA2	132 ± 13 a	21.2 ± 1.6	4.0 ± 0.4	7.0 ± 0.5	61.2 ± 1.3	32.9 ± 0.7
	G4**	10	HbA + HbF	158 ± 5 b	25.1 ± 2.5	4.0 ± 0.4	7.9 ± 0.7	62.2 ± 1.7	31.5 ± 0.6
7	G1	10	HbA + HbF + HbA2	215 ± 25 a	31.62 ± 1.2 a	4.8 ± 0.2 a	10.5 ± 0.4 a	66 ± 2.4 a	33.1 ± 0.7 a
	G2	9	HbA + HbF + HbA2	197 ± 12 a	32.8 ± 1.2 a	4.5 ±0.4 a	10.9 ± 0.4 a	72.5 ± 4.2 a	33.4 ± 0.5 a
	G3	9	HbA + HbF	145 ± 17 b	31.9 ± 2.7 a	4.3 ± 0.5 a	10.4 ± 0.9 a	73.9 ± 4.6 a	32.6 ± 0.7 b
	G4**	10	HbA + HbF + HbS	113 ± 15 c	19.0 ± 2.2 b	3.9 ± 0.5 b	5.6 ± 0.9 b	48.9 ± 1.3 b	30.7 ± 1.2 b
14	G1	10	HbA + HbF + HbA2	198 ± 22 a	36.0 ± 2.4 a	6.0 ± 0.3 a	11.8 ± 0.7 a	60 ± 2.4 a	32.7 ± 0.4 a
	G2	9	HbA + HbF + HbA2	175 ± 14 a	37.6 ± 1.9 a	5.7 ± 0.3 a	12.0 ± 0.6 a	66.3 ± 2.6 b	32.0 ± 0.3 a
	G3	9	HbA + HbF + HbA2	142 ± 4 a	33.7 ± 1.8 b	5.0 ± 0.4 a	10.7 ± 0.5 b	67.7 ± 4.7 b	31.5 ± 1.3 a
	G4**	10	HbA + HbS	87 ± 16 a	15.3 ± 2.5 c	3.6 ± 0.5 b	5.3 ± 1.0 c	42.6 ± 1.3 c	34.5 ± 1.5 b
21	G1	10	HbA + HbF + HbA2	153 ± 10 a	38.8 ± 1.9 a	6.2 ± 0.4 a	12.2 ± 0.7 a	62.2 ± 2.9 a	31.3 ± 0.5
	G2	9	HbA + HbF + HbA2	163 ± 21 a	38.5 ± 1.0 a	6.6 ± 0.2 a	11.9 ± 0.4 a	58.3 ± 1.8 a	30.9 ± 0.2
	G3	9	HbA+ HbF	139 ± 18 a	41.6 ± 2.3 b	6.6 ± 0.3 a	12.9 ± 0.6 a	63 ± 1.8 a	30.9 ± 0.4
	G4**	10	HbA	61 ± 16 a	17.1 ± 3.2 c	4.3 ± 0.8 a	5.40 ± 1 b	40.2 ± 2.0 b	31.6 ± 1.2

* The experimental condition of groups was classified as G1 = cement pen floor with iron dextran supplementation, G2 = clay pen floor with iron dextran supplementation, G3 = clay pen floor with sterile injectable saline 0.85%, G4 = cement pen floor with sterile injectable saline 0.85%; ** the group δ induced iron deficiency anemia experimentally; [I] Types of hemoglobin were obtained by agarose electrophoresis pH 8.6 and analyzed by comparison with human standard HbAS (sickle cell disease trait), [II] reference values of serum iron according to Kaneko et al. (2008), [III] reference values for erythrocyte parameters according to Thorn (2012); Different letters in the same row indicate differences between the groups at the same moment by the Tukey test (p<0.05).

intravascular increase of free hemoglobin (Kempe et al. 2006, Lang et al. 2006).

The decrease in iron levels presented a positive correlation with the absence of HbA2 band migration in the electrophoresis (ρ: 0.156, *p*=0.003). On D3, only G4 piglets presented the trace for HbA2, and from D7, all groups presented the trace for HbA2 at some point of the study except the δ group which did not present the trace at any experimental moment (Table 2).

The reduction in the amount of hemoglobin during IDA may reduce the percentage of hemoglobin subgroups as HbA2 (Keramati & Maybodi 2007), which in humans with α-thalassemia trait may present low or absent (Bain 2006), and until the present moment, there is no report of this type of hemoglobinopathy occurrence in domestic animals (Ferraz & Murao 2007, Denic et al. 2013).

In IDA, the production of HbA (α2β2) may be increased compared to HbA2, due to: 1) decrease of translation and transcription of the δ2 hemoglobin by genetic silencing; 2) The high affinity of β chains by iron molecules; 3) Higher prevalence of β chains in erythrocytes, since under normal conditions the ratio of β: δ in normal erythrocytes is 49:1; 4) HbA has a synthesis more stoichiometric stable than other subtypes; 5) Instability and lower expression of d-globin mRNA during iron deficiency (El-Agouza et al. 2002, Bain 2006, Amid et al. 2015, Steinberg & Rodgers 2015).

The absence of HbA2 in piglets of the G4 group was associated with an iron deficiency from the D7, once the supply of iron is limited, the chains of β-globin and α-globin receive a greater contribution of this mineral concerning the δ-globin chains, resulting in lower amounts of HbA2 (Passarello et al. 2012).

Thus, in comparison with the groups which did not present low serum iron results and previous studies in humans (El-Agouza et al. 2002, Keramati & Maybodi 2007, Denic et al. 2013), the reduction of HbA2 can be a marker of iron deficiency along with ferric dosage (e.g., serum iron and/or ferritin). However, it is important to highlight that the iron deficiency may not be sufficiently severe or prolonged to reduce the level of HbA2 significantly (Passarello et al. 2012).

IDA studies in humans detected that HbA2 levels were correlated to ferritin levels, reduction in erythrocyte numbers, degree of microcytosis, and zinc protoporphyrin. Therefore, the lower levels of these variables, the greater the decrease in subtype A2 (Harthoorn-Lasthuizen et al. 1999, Amid et al. 2015), corroborating the results of the present study, which pointed to the significant correlation between low levels of iron and anemia with absence HbA2 trait in piglets (Group G4) when compared to non-ferropenic groups (G1, G2, and G3).

Heterozygous beta-thalassemia humans with or without IDA may have increased expression of HbA2 when compared to IDA non-thalassemia individuals, i.e., patients, who only have mineral deficiency the amount of hemoglobin is lower exclusively due to iron deficiency (Madan et al. 1998) and when treated with exogenous iron supplementation,

HbA2 increases to normal levels (El-Agouza et al. 2002, Keramati & Maybodi 2007, Denic et al. 2013) indicating the potential of iron interference in the HbA2 production (Passarello et al. 2012), as observed in the present study in electrophotography of the ferropenic piglets (G4 group).

CONCLUSIONS

To the best of our knowledge, this is the first report of determining the hemoglobin pattern of piglets with iron deficiency anemia on electrophoreses technique, since the HbA2 trait was absent from the D7 moment onwards in comparison to the non-ferropenic groups. The low levels of iron serum were positively correlated to the absence of the HbA2 trait in the group with iron deficiency anemia (IDA).

Through our results, the measurement of HbA2 by electrophoresis can be used as an auxiliary technique for the diagnosis of iron deficiency in suckling piglets (in association with erythrogram and measurement of serum iron). However, more studies are needed to validate this technique.

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