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Use of haemoglobin analysis to evaluate anaemia status of piglets after weaning

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Introduction

Weaning is a key period involving different stress components (nutritional, immune and behavioral stress) with a negative nutrient balance which could lead to anaemia. A haemoglobin monitoring was undertaken to evaluate the anaemia status after weaning and to assess the effects of a stress factor and of a nutritional supplementation.

Material and methods

Experimental design

- A total of 360 piglets (8.35 ± 1.62 kg) weaned at 28 days were allocated to 4 groups and 24 single-sex pens for a 41-day experiment. All piglets had received 200 mg iron by IM injection at 3 days of age.
- Two factors were investigated in a 2×2 factorial design:
  - vaccination after weaning (day 1) against porcine circovirus type II (PCV2) used to induce stress (stress group vs. control group).
  - a high antioxidant phase 1 diet (HAD) providing (from weaning to day 14) 0.10 mg Se as selenium selenite, 0.20 mg Se as selenium yeast (ALKOSEL®, Lallemand Animal Nutrition, Canada), 100 mg vitamin E (α-tocopheryl acetate) and 30 mg of a Superoxide Dismutase rich melon concentrate (MELOFEED®, Lallemand SAS, France) per kg of diet vs. a low antioxidant diet (LAD) providing 0.10 mg Se as selenium selenite and 16 mg vitamin E per kg (NRC 2012 levels).

Measured parameters and statistical analyses

- A total of 40 piglets (8.15 ± 0.46 kg) were selected for haemoglobin (Hb) monitoring within the 8 pens of the medium weight class : i.e. 5 pigs close to the mean weight of the pen within one pen per treatment and sex. Capillary blood samples were obtained from the ear at days 1, 5, 9 and 34 (Hb1 to 34). The pre-weaning status of the group was evaluated 1 day before weaning (Hb-1) on 20 piglets randomly chosen in the farrowing house. Analysis were carried out in the barns using a point-of-care quantitative haemoglobin reader (HemoCue Hb201+). All piglets were weighed (n = 45 piglets/sex/treatment) at days 0, 14, 28 and 41 and average daily gain was calculated (phase 1 and phase 2 periods).
- One-way and multivariate analysis of Hb levels were performed by using a GLM model (SAS 9.4, SAS Inst., Cary, NC), with the effects of diet, vaccination, sex, parity of the sow, and farrowing or lactating litter origin, and means were compared using the t test. Data were also analyzed for repeated measures using the MIXED procedure in SAS. Prediction equations of Hb blood values from nutritional, stress and litter parameters were developed by linear regression analysis, using the REG procedure of SAS with the maxR method.

Results and discussion

❶ The reliability of the method was evaluated by repeating 60 measurements immediately on the same animals. The correlation between the two repetitions of the test is 0.6 which is a questionable internal consistency as indicated by the Cronbach's alpha table. Indeed, if the mean of all absolute pairwise differences was equal to 6.4 %, it exceeded 10 % for 15 % of the animals (Fig. 1). An instrument designed for on-field haemoglobin analysis in the real time is a useful method for rapid diagnosis but may require repetitions or a large number of animals in experiments. Although some measurements were not repeated, our study produced some interesting results.

❷ The average haemoglobin level of piglets at d 1 was 10.09 ±1.45 g/dl (Fig.2) and tended to be influenced by their lactation litter (P = 0.06). No effects of sex, birth litter, parity of the sow or vaccination were found (P > 0.15), whereas higher concentrations appeared for piglets of the HAD group (P = 0.01) or with low weaning weights (P = 0.07). These day-1 differences could have been partly explained by the pre-weaning values, but these were not measured on the same group of pigs (Hb -1: 10.59 ±1.20 g/dl).

❸ The percentage of weaned piglets with anaemia (blood Hb < 9 g/dl) peaked at d 5 (25 %), whereas this proportion was only 10 % for suckling piglets (Fig. 3). Hb5, Hb9 and Hb34 were 10.18 ±1.56, 10.38 ±1.05 and 10.35 ±0.82 g/dl, respectively. Hb34 was influenced by the effects of birth litter (P = 0.03) and sow parity (P = 0.05).

❹ At d 5 and 9, step regressions show ranked influences of Hb1 (or Hb5), weaning weight and daily gain (from d 0 to 14). At d 34, haemoglobin level was influenced by d 0 to 14 daily gain, sex, parity number, Hb1 (Fig. 4).

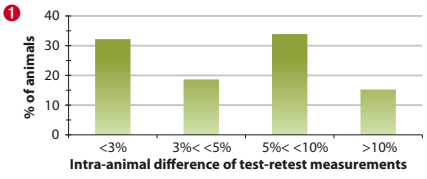
❺ Piglets fed with the HAD diet had better haemoglobin values at d 1 (P < 0.01), 5 (P = 0.09) and 9 (P = 0.10), and for the whole period (repeated measures analysis; P < 0.01). It is apparent that fewer piglets had anaemia (<9 g dl) with the anti-oxidant supplementation than with the LAD diet at d 5 (15 vs 35%) and 9 (0 vs 20 %) (Fig. 5).

❹ *Regression analysis for Hb at days 1, 5, 9 and 34 (g/dl) using nutritional, stress and litter parameters as predictors (n=40)*

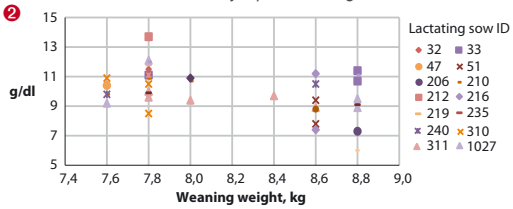
Dependent variable	Step	Regression equation (MaxR method)	R_2	RSD
Hb1	1	21.8 (±3.76)[a] - 1.44 (±0.46) BW_1[b]	0.20	1.33
Hb5	1	3.67 (±1.38) + 0.67 (±0.14) Hb1	0.40	1.24
	2	13.3 (±4.57) - 1.04 (±0.46) BW_1 + 0.53 (±0.14) Hb1	0.47	1.18
	3	13.9 (±4.49) - 1.05 (±0.45) BW_1 + 0.57 (±0.14) Hb1 - 0.004 (±0.002) ADG_{14}	0.51	1.16
Hb9	1	7.09 (±0.99) + 0.33 (±0.10) Hb5	0.23	0.95
	2	8.59 (±0.99) - 0.005 (±0.002) ADG_{14}+ 0.31 (±0.09) Hb5	0.41	0.84
	3	14.1 (±3.51) - 0.56 (±0.34) BW_1- 0.005 (±0.002) ADG_{14}+ 0.23 (±0.10) Hb5	0.45	0.83
Hb34	1	9.11 (±0.42) + 0.005 (±0.001) ADG_{14}	0.21	0.75
	2	8.50 (±0.54) + 0.40 (±0.23) Sx - 0.005 (±0.001) ADG_{14}	0.27	0.73
	3	8.29 (±0.55) + 0.43 (±0.23) Sx + 0.08 (±0.05) Parity + 0.004 (±0.001) ADG_{14}	0.31	0.72
	4	9.50 (±0.94) + 0.38 (±0.23) Sx + 0.09 (±0.05) Parity - 0.13 (±0.08) Hb1 + 0.004 (±0.001) ADG_{14}	0.36	0.70

a Values in parentheses are standard errors. b BW1: body weight at d 1, ADG14: average daily gain from d 0 to 14; Hb1, 5 : blood haemoglobin concentration at d 1 or 5; Sx = sex (1 for barrow, 2 for female); R2: R-square; RSD: residual standard deviation. Minimum criteria for inclusion of independent effect in the model is P < 0.15.

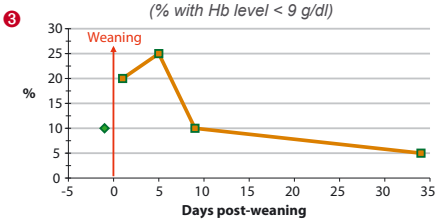
❶ *Distribution of the Hb measurement repeatability*



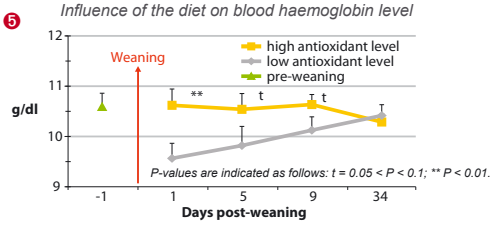
❷ *Influence of litter and weaning weight on piglet haemoglobin blood level at day 1 post weaning*



❸ *Evolution of post-weaning anaemia in piglets (% with Hb level < 9 g/dl)*



❺ *Influence of the diet on blood haemoglobin level*



Conclusion

Haemoglobin (Hb) monitoring may be useful to evaluate the individual nutritional status of weaned piglets, and repeating the measurements should reduce variability. Immediately after weaning, the Hb concentration tended to be influenced by each pig's lactation litter of origin and to be decreased with heavier weaning weight. Five days after weaning, the percentage of piglets with anaemia (Hb < 9 g/dl) was maximized and Hb concentration was then increased by weight gain. Lastly, an antioxidant supplementation in the feed appeared to increase Hb values after weaning.

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