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Effect of chromium picolinate supplementation before weaning on the hematological profile, behaviour and weaning stress in piglets

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Abstract: Weaning is one of the most critical periods in pig production, inducing profound physiological, metabolic, immunological and behavioural changes that may adversely affect piglet welfare and performance. The present study evaluated the effects of early chromium picolinate supplementation on hematological and biochemical blood parameters, differential leukocyte counts, behavioural responses and physiological adaptation to weaning stress. Thirty-two piglets originating from eight sows were allocated to a control group and an experimental group (16 animals each). Piglets in the experimental group received 200 μg chromium picolinate per animal per day, starting five days before weaning and continuing until the third day after weaning, whereas the control group received the basal diet only. Blood samples were collected before supplementation, on the day of weaning and at the end of the experimental period. Behavioural observations were performed during the first two days after weaning. Chromium picolinate supplementation did not significantly affect glucose concentration or the main hematological indicators ($P>0.05$). A significant additive effect was observed for eosinophils ($P = 0.030$), while the sampling period significantly affected glucose, leukocyte, erythrocyte, banded and segmented neutrophil, and lymphocyte values. During the first post-weaning day, supplemented piglets showed lower locomotor activity and longer resting and feeding times than controls. These findings suggest that supplementation initiated before weaning may support behavioural adaptation and help moderate selected stress-related changes without adversely affecting hematological homeostasis.

Keywords: chromium picolinate; piglets; weaning stress; behaviour; hematology; differential leukocyte count

INTRODUCTION

Weaning represents one of the most challenging periods in pig production, exposing piglets to abrupt nutritional, environmental and social changes that trigger complex physiological, immunological and behavioural responses. Separation from the sow, dietary transition from milk to solid feed, regrouping and adaptation to a new environment frequently result in reduced feed intake, impaired intestinal function, increased susceptibility to disease and decreased growth performance (Campbell et al., 2013; Salak-Johnson and Webb, 2018).

Nutritional strategies aimed at alleviating weaning-associated stress have therefore received considerable scientific attention. Among these, trace minerals play a pivotal role because of their involvement in numerous enzymatic, metabolic and antioxidant processes. Although required in minute quantities, trace elements are essential for maintaining immune competence, cellular integrity and normal growth. They function as cofactors for enzymes involved in antioxidant defence, energy metabolism and physiological homeostasis, thereby contributing to resistance against environmental and physiological stressors (Yatoo et al., 2013).

Chromium is involved in carbohydrate, lipid and protein metabolism through its capacity to enhance insulin action and glucose utilization (National Research Council, 1997). Trivalent chromium (Cr^{3+}) is the form most common-

ly used in animal nutrition. Organic chromium sources, particularly chromium picolinate, have attracted interest because of their bioavailability and possible effects on stress resistance, immune function and productive performance. Studies in pigs have reported variable responses, depending on chromium source, dose, age and stress intensity (van Heugten and Spears, 1997; Liu et al., 2017; He et al., 2023).

From a physiological perspective, weaning stress activates the hypothalamic-pituitary-adrenal axis, resulting in increased glucocorticoid secretion, particularly cortisol. Sustained cortisol elevation exerts catabolic effects, suppresses immune function and alters carbohydrate, lipid and protein metabolism (Martínez-Miró et al., 2016; Salak-Johnson and Webb, 2018). These endocrine responses are accompanied by changes in hematological and biochemical blood parameters, which can serve as objective indicators of physiological status and stress. Behavioural alterations, including increased locomotion, reduced resting time and disrupted feeding, are also sensitive indicators of impaired adaptation during the immediate post-weaning period.

Despite the increasing number of studies on chromium supplementation in pigs, information on supplementation initiated before weaning and continued through the immediate post-weaning period remains limited. Most studies have focused on growth performance and carcass traits, whereas behavioural adaptation and detailed stress-related hematological responses during this critical transition have received less attention.

Therefore, the objective of the present study was to evaluate the effects of chromium picolinate supplementation initiated before weaning on blood glucose, hematological parameters, differential leukocyte profile, behavioural responses and physiological adaptation to weaning stress in piglets.

MATERIALS AND METHODS

Animals, housing and experimental design

The study was conducted at the Experimental Farm of the Agricultural Institute - Shumen, Bulgaria. Thirty-two clinically healthy piglets originating from eight sows were included. Four piglets from each litter (two males and two females) were selected and balanced for origin, sex, age and live weight according to the analogue method. The piglets were allocated to two treatments, each comprising 16 animals: a control treatment receiving only the basal diet and an experimental treatment receiving chromium picolinate at 200 μg per animal per day.

Piglets were weaned at 35 days of age. After weaning, they were transferred to the nursery unit and housed in four elevated pens, with eight piglets per pen. The animals received a pelleted complete feed formulated for the respective age category according to the feeding programme of the Experimental Farm. Drinking water was available ad libitum through nipple drinkers. General health status was checked and recorded daily throughout the experimental period.

Chromium picolinate supplementation began five days before weaning, while the piglets were still housed with the sow, and continued until the third day after weaning. The daily amount was administered individually to ensure consumption of 200 μg chromium picolinate per piglet.

Blood sampling and laboratory analyses

Blood samples were collected on three occasions: before the start of supplementation, on the day of weaning and at the end of the experimental period. Samples were analyzed for blood glucose and selected hematological indicators. Total leukocyte count (WBC), erythrocyte count (RBC), haemoglobin concentration (HGB) and platelet count (PLT) were determined using an Abacus automated hematology analyzer. Blood glucose was measured using an Olympus biochemical analyzer.

Differential leukocyte counts were determined manually by a visual-optical method and included banded neutrophils, segmented neutrophils, eosinophils, monocytes and lymphocytes. All blood sampling and laboratory procedures were performed consistently across treatment groups and sampling occasions.

Behavioural observations

Behaviour was recorded during the first two consecutive days after weaning. Direct observations were conducted for 10 h per day using scan sampling at 2-min intervals. The behavioural categories were locomotion, lying and feeding. Water intake was also monitored during observation, and room temperature was recorded hourly. Behavioural data were summarized as absolute duration (min) and as a proportion of the total observation time for each sex-by-treatment subgroup.

Statistical analysis

Data were processed using Microsoft Excel 2016 and Minitab 16. Descriptive statistics included the mean, variance and standard error. Differences among the four sex-by-treatment subgroups at each sampling occasion were evaluated by analysis of variance. In addition, a multifactorial ANOVA model was used to estimate the main effects of chromium picolinate supplementation (additive), sex and sampling occasion on the biochemical, hematological and differential leukocyte variables. Tukey's post-hoc test was applied when appropriate. Statistical significance was accepted at $P<0.05$; values between $P = 0.05$ and $P<0.10$ were interpreted as statistical tendencies. Behavioural results are presented descriptively because inferential statistics for repeated scan observations were not available.

RESULTS AND DISCUSSION

Blood glucose and hematological profile

The descriptive results for blood glucose and the main hematological indicators are presented in Table 1, while the P values for the main effects of additive, sex and sampling occasion are summarized in Table 2. Chromium picolinate supplementation did not significantly affect glucose, WBC, RBC, HGB or PLT ($P>0.05$; Table 2). Sampling occasion, however, significantly affected glucose ($P<0.001$), WBC ($P=0.006$) and RBC ($P=0.003$), indicating that the transition from suckling to weaning was the dominant source of variation for these variables.

Blood glucose declined from the initial measurement to the end of the trial in all subgroups. On the day of weaning, the lowest mean value was observed in experimental females (6.25 mmol/L), but differences among the four subgroups were not significant ($P = 0.491$; Table 1). The significant sampling effect reflects the metabolic adjustment associated with the transition from milk to solid feed rather than a direct hypoglycaemic effect of chromium. Similar absence of a consistent effect on circulating glucose has been reported in pigs supplemented with organic chromium (Untea et al., 2017; Edmonds et al., 2024).

WBC values increased around weaning, with the highest mean observed in experimental males on the day of weaning (19.98 $\times 10^9$/L), but treatment differences were not significant. The significant sampling effect is compatible with a transient stress-related leukocyte response. Weaning can alter leukocyte trafficking through cortisol- and catecholamine-mediated mechanisms (Martínez-Miró et al., 2016; Salak-Johnson and Webb, 2018). The absence of an additive effect indicates that the applied dose did not produce detectable leukocytosis or leukopenia.

RBC increased between the initial and subsequent samplings, whereas HGB remained comparatively stable. Chromium picolinate did not significantly affect either variable, which agrees with reports that physiologically relevant chromium doses do not consistently alter erythropoiesis or haemoglobin concentration in pigs (Kim et al., 2009). A tendency for a sex effect was observed for HGB ($P = 0.089$), but the magnitude and biological relevance of this tendency should be interpreted cautiously.

PLT showed pronounced within-group variability and was not significantly influenced by additive, sex or sampling occasion. High individual variability in platelet counts has been described in young pigs and can reduce the ability to detect dietary effects (Pliszczak-Król et al., 2016). Overall, the results indicate that supplementation at 200 μg per animal per day did not adversely affect the principal hematological variables measured in the present study.

Table 1. Blood glucose and hematological parameters of piglets at the beginning of the experiment, on the day of weaning and at the end of the experiment

Indicator	Date of blood sampling	Treatments												P-value
		Control ♂ (CM)*			Experimental ♂(EM)*			Control ♀(CF)*			Experimental ♀(EF)*			
		Mean	Variance	SE	Mean	Variance	SE	Mean	Variance	SE	Mean	Variance	SE	
Glucose (Glu) (mmol/l)	Beginning of the exp.	7.75	1.07	0.45	8.71	1.14	0.48	8.37	0.42	0.29	7.51	0.32	0.25	0.143
	On the day of weaning	6.79	0.22	0.12	6.82	0.52	0.32	6.87	0.51	0.32	6.25	0.73	0.38	0.491
	End of the experiment	6.41	0.44	0.29	6.72	0.58	0.34	6.20	0.01	0.05	5.88	0.35	0.26	0.180
Leukocytes (WBC) (x10^9/L)	Beginning of the exp.	12.90	8.71	1.07	15.24	9.75	1.40	11.96	10.19	1.43	12.77	8.30	1.29	0.349
	On the day of weaning	14.76	25.94	2.28	19.98	2.38	1.57	16.92	22.49	2.12	18.30	28.96	2.41	0.606
	End of the experiment	16.24	7.39	1.22	16.78	0.84	0.41	17.08	17.51	1.87	17.00	12.44	1.58	0.973
Erythrocytes (RBC) (x10^12/L)	Beginning of the exp.	5.68	0.04	0.03	5.75	0.15	0.18	5.74	0.29	0.24	5.98	0.43	0.29	0.767
	On the day of weaning	6.50	0.23	0.21	6.23	0.21	0.20	5.96	0.40	0.28	6.29	0.32	0.25	0.494
	End of the experiment	6.36	0.18	0.19	6.42	0.09	0.13	6.04	0.08	0.13	6.35	0.58	0.34	0.611
Haemoglobin (HGB) (g/L)	Beginning of the exp.	105.60	527.3	10.27	104	236.5	6.88	99.2	543.7	10.43	106.60	282.8	7.52	0.938
	On the day of weaning	111.00	153.5	5.54	106	149.5	5.47	99	413.5	9.09	101.4	67.8	3.68	0.557
	End of the experiment	107.2	224.7	6.7	105.8	199.7	6.32	97.6	244.8	7	101.6	201.3	6.35	0.732
Platelets (PLT) (x10^9/L)	Beginning of the exp.	646.4	43.631	93.41	684.4	31.663	79.57	642.4	40.741	90.26	690.4	56.378	106.18	0.974
	On the day of weaning	666.75	73.742	135.78	687.5	23.406	76.50	843.5	86.031	146.66	802	57.334	119.72	0.693
	End of the experiment	561	57.396	119.79	670.25	82.17	45.32	758.25	124.45	176.39	785.75	46.796	108.16	0.573

**Notice: CM: control males; EM: experimental males; CF: control females; EF: experimental females; SE: standard error. Experimental piglets received 200 μg chromium picolinate per animal per day. Means within a row sharing a common superscript letter do not differ significantly (P>0.05).*

Source: Authors' own elaboration

Table 2. P-values for the effects of chromium picolinate supplementation, sex and sampling occasion on blood glucose and hematological parameters

Variable	Additive	Sex	Sample
Glucose, mmol/L	0.689	0.072	<0.001
WBC*, $\times 10^9$/L	0.351	0.576	0.006
RBC*, $\times 10^{12}$/L	0.339	0.461	0.003
HGB*, g/L	0.756	0.089	0.851
PLT*, $\times 10^9$/L	0.534	0.206	0.609

**Notice: WBC: leukocytes; RBC: erythrocytes; HGB: haemoglobin; PLT: platelets.*

Source: Authors' own elaboration

Differential leukocyte profile

Differential leukocyte data are shown in Table 3, and the corresponding multifactorial ANOVA results are presented in Table 4. Sampling occasion significantly affected banded neutrophils (P = 0.008), segmented neutrophils (P<0.001) and lymphocytes (P<0.001). In contrast, chromium picolinate exerted a significant main effect only on eosinophils (P = 0.030).

On the day of weaning, segmented neutrophils increased and lymphocytes decreased, particularly in males. In control males, segmented neutrophils rose from 26.0% to 40.8%, while lymphocytes decreased from 63.8% to 50.0%. Comparable changes were observed in experimental males. The significant sampling effects therefore indicate a general response to weaning rather than a treatment-specific effect. The reciprocal pattern of neutrophilia and lymphopenia is consistent with activation of the hypothalamic-pituitary-adrenal axis and redistribution of circulating leukocytes during acute stress (Martínez-Miró et al., 2016).

The additive effect on eosinophils was statistically significant (P = 0.030). Across the whole period, experimental males had a mean eosinophil proportion of 2.00%, compared with 1.07% in control males; the corresponding means in females were 1.80% and 1.73%. On the day of weaning, eosinophils declined to 0.8% in control males but remained at 1.8% in experimental males and females. Because eosinophil values can decrease during glucocorticoid-mediated stress, these results suggest that chromium supplementation may have moderated this component of the stress response. Nevertheless, the modest sample size and the absence of cortisol measurements require cautious interpretation.

Banded neutrophils and monocytes remained within a narrow range and were not affected by supplementation. The absence of marked band neutrophilia or a sustained monocyte increase supports the daily clinical observations that no acute infectious episode occurred during the trial. Taken together, the differential count indicates that weaning was the principal determinant of neutrophil and lymphocyte dynamics, whereas chromium picolinate was associated with a more stable eosinophil profile.

Behavioural adaptation during the first two days after weaning

The behavioural distribution during the first and second days after weaning is presented in Tables 5 and 6 and Figures 1 and 2. The values are descriptive and should therefore be interpreted as patterns rather than as statistically confirmed treatment effects.

During the first day after weaning, locomotion accounted for 31% and 36% of the observation time in control males and females, respectively, compared with 21% and 19% in experimental males and females. At the same time, supplemented piglets spent more time lying (47-48% vs. 35-42% in controls) and feeding (31-34% vs. 27-29% in controls). This pattern is compatible

Table 3. Differential leukocyte profile of piglets at the beginning of the experiment, on the day of weaning and at the end of the experiment

Indicator	Date of blood sampling	Treatments												P-value
		Control ♂ (CM)*			Experimental ♂(EM)*			Control ♀(CF)*			Experimental ♀(EF)*			
		Mean	Variance	SE	Mean	Variance	SE	Mean	Variance	SE	Mean	Variance	SE	
Banded neutrophils, (St), %	At the beginning of the experiment	1.2	0.7	0.37	1	0.5	0.32	0.4	0.8	0.4	1.2	2.7	0.73	0.618
	On the day of weaning	1.8	4.7	0.97	1.4	1.3	0.51	2.2	1.2	0.49	2.6	2.3	0.68	0.648
	At the end of experiment	2	2	0.63	1.6	0.3	0.24	2.6	2.3	0.68	2.2	0.7	0.83	0.592
	For the hole period	1.67	2.24	0.39	1.33	0.67	0.21	1.73	2.21	0.38	2	2	0.37	0.596
Segmented neutrophils, (Sg), %	At the beginning of the experiment	26	45.5	3.02	27.8	11.2	1.50	24	50.5	3.18	27	11.2	2.65	0.770
	On the day of weaning	40.8	46.2	3.04	42.4	63.3	3.56	29.4	140.8	5.31	35	26	2.28	0.093
	At the end of experiment	32.8	23.2	2.15	32	18.5	1.92	35.2	15.7	1.77	35.2	29.2	2.42	0.687
	For the hole period	33.2	72.03	2.19	34.01	66.92	2.11	29.53	81.55	2.33	31.4	37.52	1.58	0.427
Eosinophils, (Eo), %	At the beginning of the experiment	1.2	0.2	0.2	2.2	1.2	0.49	2	1	0.45	1.8	0.7	0.37	0.340
	On the day of weaning	0.8	0.7	0.37	1.8	1.2	0.49	1.4	0.8	0.4	1.8	1.7	0.58	0.412
	At the end of experiment	1.2	0.2	0.2	2	1	0.45	1.8	0.2	0.2	1.8	0.7	0.37	0.362
	For the hole period	1.07ab	0.35	0.15	2a	1	0.26	1.73	0.64	0.21	1.8b	0.89	0.24	0.023
Monocytes (Mo), %	At the beginning of the experiment	7.8	0.7	0.37	6.6	3.8	0.87	7.8	2.7	0.73	8	1	0.45	0.418
	On the day of weaning	6.6	0.8	0.4	7.8	0.7	0.37	7.4	5.3	1.03	7.2	2.2	0.66	0.652
	At the end of experiment	5.6	13	0.51	8	7	1.8	7.8	3.2	0.8	6.4	2.8	0.75	0.180
	For the hole period	6.67	1.67	0.33	7.47	3.70	0.50	7.67	3.24	0.46	7.2	2.17	0.38	0.379
Lymphocytes (Ly), %	At the beginning of the experiment	63.8	52.7	3.25	62.4	32.3	2.54	65.8	39.2	2.8	62	49	3.13	0.797
	On the day of weaning	50	65.6	3.62	46.6	68.3	3.7	59.6	97.8	4.42	53.4	32.8	2.56	0.113
	At the end of experiment	58.4	19.8	1.99	56.4	34.8	2.64	52.6	39.3	2.8	57.4	28.3	2.38	0.397
	For the hole period	57.4	73.97	2.22	55.13	84.12	2.37	59.33	81.52	2.33	57.6	44.69	1.73	0.601

**Notice: CM: control males; EM: experimental males; CF: control females; EF: experimental females; SE: standard error. Experimental piglets received 200 μg chromium picolinate per animal per day. Means within a row sharing a common superscript letter do not differ significantly (P>0.05).*

Source: Authors' own elaboration

Table 4. P-values for the effects of chromium picolinate supplementation, sex and sampling occasion on differential leukocyte counts

Variable	Additive	Sex	Sample
Banded neutrophils, %	0.918	0.257	0.008
Segmented neutrophils, %	0.430	0.071	<0.001
Eosinophils, %	0.030	0.304	0.431
Monocytes, %	0.700	0.399	0.528
Lymphocytes, %	0.274	0.230	<0.001

Source: Authors' own elaboration

Table 5. Absolute (minutes) and relative (%) distribution of behavioural reactions in control and chromium-supplemented piglets on observation day 1

Group	Unit	Behavioural reactions		
		Locomotion	Lying	Feeding
CM*	min	1186	1604	1050
	%	31	42	27
EM*	min	812	1854	1174
	%	21	48	31
CF*	min	1372	1340	1128
	%	36	35	29
EF*	min	746	1798	1296
	%	19	47	34

**Notice: CM: control males; EM: experimental males; CF: control females; EF: experimental females*

Source: Authors' own elaboration

Table 6. Absolute (minutes) and relative (%) distribution of behavioural reactions in control and chromium-supplemented piglets on observation day 2

Group	Unit	Behavioural reactions		
		Movement	Lying	Feeding
CM*	min	1048	1764	1028
	%	27	46	27
EM*	min	874	1964	1002
	%	23	51	26
CF*	min	734	1716	1390
	%	19	45	36
EF*	min	1114	1946	780
	%	29	51	20

**Notice: CM: control males; EM: experimental males; CF: control females; EF: experimental females*

Source: Authors' own elaboration

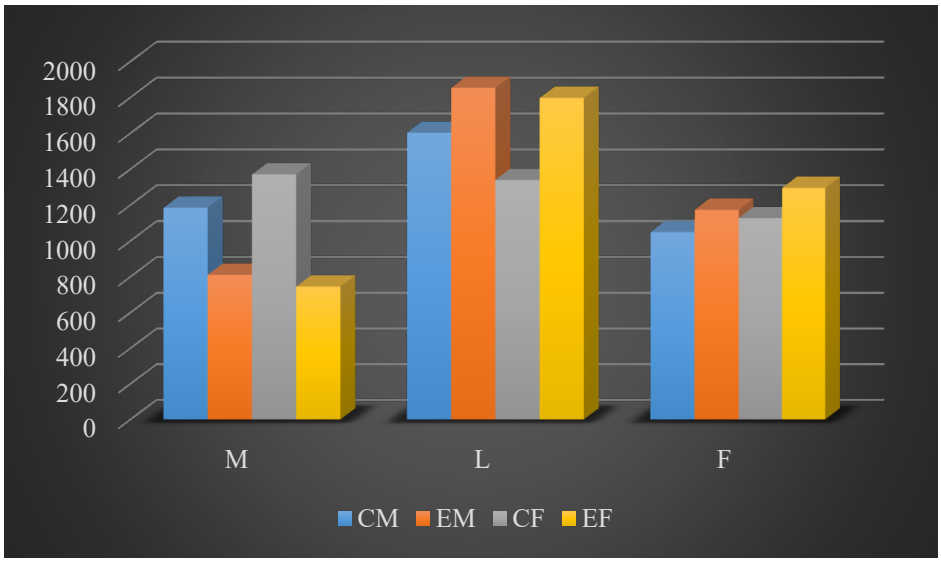

Figure 1. Time spent in movement, lying and feeding by control and chromium-supplemented piglets on observation day 1

Legend: M – Movement; L – Lying; F – Feeding; CM/CF – Control Males/Females; EM/EF – Experimental Males/Females

Source: Authors' own elaboration

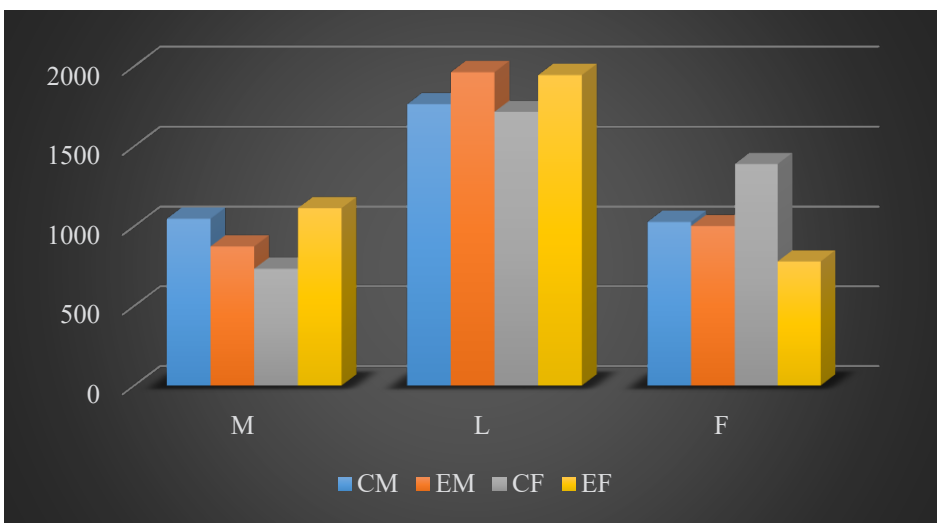

Figure 2. Time spent in movement, lying and feeding by control and chromium-supplemented piglets on observation day 2

Legend: M – Movement; L – Lying; F – Feeding; CM/CF – Control Males/Females; EM/EF – Experimental Males/Females

Source: Authors' own elaboration

with a less pronounced immediate post-weaning disturbance, because excessive locomotion after separation and relocation may reflect searching behaviour, arousal and difficulty settling in the new environment (Van Kerschaver et al., 2023).

On the second day, behavioural differences were less uniform. Experimental males continued to show more lying and less locomotion than control males. Experimental females, however, showed increased locomotion (29%), high lying

time (51%) and lower feeding time (20%) than control females. Thus, the behavioural findings do not support a uniform treatment effect across both sexes and both days. Instead, the clearest treatment-associated pattern occurred during the first 24 h after weaning, when both supplemented subgroups displayed lower locomotion and greater resting and feeding activity.

Resting behaviour can indicate that animals have settled in the new environment, provided it is not accompanied by signs of illness or lethargy. Early feed intake is also important because post-weaning anorexia contributes to impaired intestinal function and growth (Upadhaya and Kim, 2021). In the present study, the higher proportion of feeding during the first observation day in supplemented piglets may therefore represent a favourable adaptive response. However, feeding duration was derived from direct scan observations and was not supported by individual feed-intake measurements; conclusions about nutritional intake must consequently remain tentative.

Overall, the combined hematological and behavioural findings suggest that chromium picolinate supplementation initiated before weaning was associated with selected signs of improved adaptation, particularly a stable eosinophil profile and a calmer behavioural pattern during the first post-weaning day. The absence of significant treatment effects on most blood variables and the descriptive nature of the behavioural data preclude stronger causal claims.

CONCLUSIONS

1. Chromium picolinate supplementation at 200 μg per animal per day did not adversely affect blood glucose, total leukocytes, erythrocytes, haemoglobin or platelets.

2. Sampling occasion, reflecting the transition through weaning, was the principal source of variation in glucose, WBC, RBC, neutrophil and lymphocyte values.

3. The significant additive effect on eosinophils and the avoidance of a marked decline in supplemented piglets suggest a possible moderation of one stress-related hematological response.

4. During the first day after weaning, supplemented piglets showed lower locomotor activity and spent more time lying and feeding than controls, indicating more rapid behavioural settling; this pattern was not consistent across both sexes on the second day.

5. The findings support further investigation of pre-weaning chromium picolinate supplementation as a nutritional strategy for improving piglet adaptation and welfare during weaning.

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