South African Journal of Animal Science 2023, 53 (No. 3)

Suckling piglets submitted to creep feeding management from 5 days-old showed optimal performance and exploratory behaviour

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(Submitted 26 July 2022; Accepted 9 January 2023; Published 17 July 2023)

Abstract
This study aimed to assess the effects of starting creep feeding at different ages on performance, behaviour, haematological, and biochemical parameters in piglets. A total of 138 piglets (1.34 ± 0.25 kg, *n* = 13 sows), weaned at 21 d-old, were assigned to treatments using a completely randomized design. The litter was divided into two groups: seven litters (*n* = 80 piglets) were subjected to creep feeding on the fifth day of age (creep 5) and five litters (*n* = 58 piglets), on the tenth day of age (creep 10). Piglets were weighed at birth, weaning, and after the first week in the nursery. Piglet behaviour was evaluated daily for 15 min until day 20. Blood was collected on day 13, at weaning, and at the end of the first week in the nursery. There was no difference in body weight in the suckling phase, but piglets fed Creep 5 showed greater body weight gain in the first nursery week. The Creep 5 group had lower feed leftovers during the five days of evaluation of feed intake. There were no differences in haematological parameters and amylase concentration; however, the piglets spent more time lying down, sucking, and massaging the udder. On day 8, the piglets in the Creep 5 group showed a higher frequency of exploratory behaviour. In conclusion, piglets fed Creep 5 had optimal performance after weaning and improved feed intake capacity by displaying more exploratory behaviour than Creep 10. Creep management did not alter haematological and biochemical parameters.

Keywords: blood parameters, body weight, creep, feeding, litter, post-weaning
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Introduction
The provision of a solid pre-weaning diet is also known as creep feeding; the main purpose is to minimize the stress of piglets when weaned and submitted to the nursery phase (Bruininx *et al.,* 2002). In this regard, Moeser *et al.* (2017) reported that an abrupt change in feed source can cause a reduction in intestinal villi because in the feed, there are immunogenic agents that can promote a local inflammatory response and compromise the intestinal architecture. This also indicates the importance of analysing the immune responses (Pluske *et al.,* 2018) to understand how feeding at different ages impacts the blood cells of the immune system. The predominant digestive enzyme in the small intestine is lactase when the piglets ingest only sow milk (Hampson & Kidder, 1986). However, there is an increase in amylase activity in the intestinal mucosa during weaning when the animal is subjected to creep feeding (Shields *et al.,* 1980), but information is limited on blood amylase concentration. In

addition, it has been shown that the early supply of feed in the suckling phase aims at maturation of the piglet digestive tract for exclusive feeding during the nursery phase (Dong & Pluske, 2007).

The start of creep feeding is indicated between five and ten days of age for the piglet, improving body weight at weaning and post-weaning (Bruininx *et al.,* 2002; Bruininx *et al.,* 2004; Pluske *et al.,* 2007; Pluske *et al.,* 2018). However, pigs naturally exhibit late investigative behaviour, in which they start to explore different ingredients sources only between 24 to 36 d-old (Petersen *et al.,* 1989).

Thus, previous studies (Bruininx *et al.,* 2002; Pluske *et al.,* 2007; Sulabo *et al.,* 2010) reported greater exploratory behaviour in piglets that consumed creep feed. Overall, if creep feed provision reduces post-weaning stress, it may also affect piglet behaviour (Middelkoop *et al.,* 2020) and consequently improve the biochemistry and immunology of the blood; but to our knowledge, this has not been studied.

Although the beneficial effects of this management are known, it is not clear what the most appropriate age is to start to improve piglet growth. Therefore, this study aimed to test the effect of starting creep feeding at different ages on the growth performance, behavioural characteristics, haematological, and biochemical parameters in piglets.

Material and methods

The ethical clearance for this research was granted by the Animal Care and Use Committee (protocol 25/2018).

A total of 138 piglets (1.34 ± 0.25 kg BW) weaned at 21 d-old, originating from 13 females (Cambrough 90, Agroceres PIC®), were classified into three to six classes in farrowing order (4.17 ± 1.27) in a completely randomized design. One sow and her litter were excluded from the experiment due to sanitary problems. The sows used in the study did not show any type of change in relation to physical health during the pre- and postpartum period. Immediately after birth, newborn piglets were subjected to drying and nasal cleaning, suckling guidance, individual weighing (with a 0.01 g precision scale), navel asepsis, and identification using sequentially numbered ear tags. The animals were housed with the sow and had free access to the creep (0.80 × 0.80 cm), which had a heating lamp and was coated with wood shavings. On the third day of age, 1 mL of toltrazuril was supplied orally and 1 mL of intramuscular iron was administered. In the case of a large litter (> 15 piglets), the uniformization of litters was performed so that all piglets had access to the sow's teats.

After initial management, the litter was divided into two groups: seven litters (*n* = 80 piglets; average = 11.42; minimum = 9 piglets/sow; maximum = 14 piglets/sow) were submitted to creep feeding management on the fifth day of age (creep 5) and five litters (*n* = 58 piglets; average = 11.60; minimum = 10 piglets/sow; maximum = 14 piglets/sow), on the tenth day of age (creep 10).

The diet (Baybipap®, Mig-Plus) was supplied daily in the amount of 50 g/litter. The leftovers were weighed for intake control and placed in specialized accessory feeders for the suckling piglets (polypropylene material, with a partition and a fixing system to the floor). At weaning and at the end of the first week of nursery, the piglets were weighed individually and handled similarly.

In the nursery, piglets were housed in pens (10 m^2; with a plastic slatted floor, two nipple water drinkers, and 0.2 m^2 of feeder space) and the density varied according to the size of the litter. Litters of the experiment were housed together (minimum number of piglets per pen = 20; maximum number of piglets per pen = 25) and were separated according to sex. Piglets were fed *ad libitum*.

The behavioural assessments of the piglets started on the fifth day of age (d5) and were completed on day 20 (d20). The behaviour was evaluated daily (14h00) using the observational method by two trained observers for 15 min in each litter and expressed as percentages of behaviour. A total of 5,372 behaviours were reported in the period, which were recorded on an ethogram sheet. The postures analysed were: standing, sitting, lying down (on the creep or near the sow) and moving; and the behavioural activities were: social interaction, fighting for teats, udder massaging, sucking, exploring (environment, feeder, or pacifier), drinking water, eating feed, urinating, defecating, and others.

On the 13th day of age, at weaning and at the end of the first week of nursery phase, the blood of three piglets per litter (light-, middle- and heavy-weight piglets) was collected. Then, blood collection (~3 mL) was performed by venipuncture in the brachial plexus using 0.38 × 13-mm gauge needles. Blood was transferred to two glass tubes containing ethylenediaminetetraacetic acid and clot activator (amylase), which were labelled, stored in a styrofoam box with ice, and sent to the Blood Parameters Laboratory. Blood samples were centrifuged at 3,000 × *g* for 5 min. Complete blood count was determined using an analyser (Sysmex® - pocH 100iV Diff) previously calibrated for pigs. Blood was analysed in relation to haematological assessments (haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, leukogram, and platelet count) and biochemistry (amylase concentration). Amylase was assessed by refractometry using Reflotron®.

Relevant performance data was submitted to the Shapiro–Wilk normality test and, as they presented a normal distribution (P >0.05), were compared using a *t*-test for independent variables. Fisher's test was used to assess the frequency of behaviour (FREQ procedure). The analysis of the feed leftovers over time was performed using the MIXED procedure. Treatment was included as fixed effect (T_i), and the effect of piglets nested within treatment ($p_{j:i}$), day of experiment (t_k), and residual error (ε_{ijk}) were included as random effects, following the general model:

$$Y_{ijk} = \mu + T_i + p_{j:i} + t_k + \beta\,(X_{ijk} - \bar{X}\ldots) + \varepsilon_{ijk},$$

in which Y_{ijk} = average observation of the dependent variable in each plot measured in the i-th treatment class, in the j-th replication, and in the k-th day of experiment; μ = effect of the overall average. Data regarding the analysis of haematological and biochemical parameters were analysed using the general linear model procedure. Piglet body weight was considered a covariate [β (X_{ijk} -$\bar{X}$...)] for the haematological parameters. The data were analysed using the Statistical Analysis System (version 9.3) program.

Results and Discussion

There was no difference (P >0.05) in body weight between the groups in the suckling phase and after the first nursery week (Table 1). However, piglets that received early feed showed (P =0.035) a greater body weight gain in the first nursery week. In creep 5, 13.75% (n = 11) of the piglets showed loss of body weight in the nursery phase. This value was greater (25.86%, n = 15; P =0.072) in creep 10.

Table 1 Averages (± standard error) of the effect of starting creep feeding at different ages on the growth performance of piglets

Parameters (kg)[1]	Experimental groups[2] Creep 5	Creep 10	*P*-value[3]
Birth weight	1.31 ± 0.241	1.38 ± 0.265	0.150
Weaning weight	5.60 ± 1.068	5.55 ± 1.142	0.808
Weight in the first week of nursery	6.17 ± 1.179	5.90 ± 1.308	0.202
DBWG in the suckling phase	0.19 ± 0.042	0.18 ± 0.044	0.505
DBWG in the first week of nursery	0.08 ± 0.088	0.05 ± 0.087	0.035

[1]DBWG = daily body weight gain; [2]Creep 5 = piglets that received solid feed from the fifth day of age; Creep 10 = piglets that received solid feed from the tenth day of age; [3]Significance level

The feed leftovers differed between the groups on days 11, 14, 15, 17, and 19 of suckling (Figure 1). The creep 5 group had lower feed leftovers, which indicated greater feed intake.

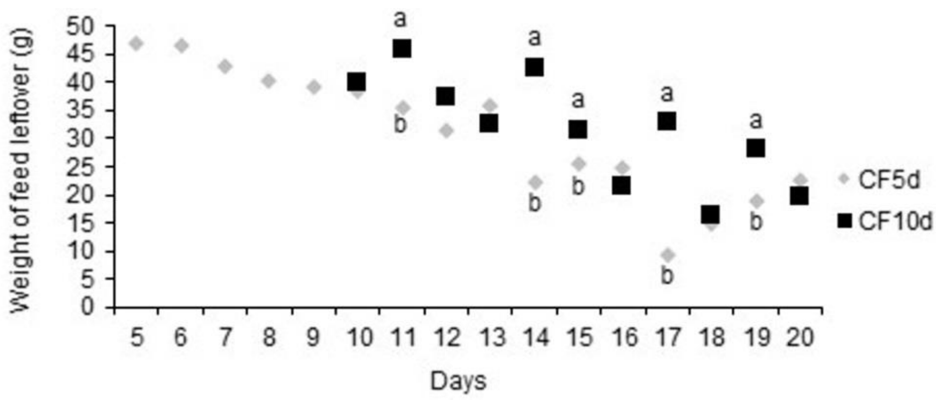

Figure 1 Weight of feed leftover in piglets treated from 5 (CF5d) and 10 (CF10d) days of age
[a,b] indicate statistical difference between groups (P <0.0001)

Our study showed that creep feeding from 5 d had a beneficial effect on feed intake and feeding behaviour. The growth performance of piglets in the nursery phase is directly related to feed intake in the suckling phase (Pajor *et al.,* 1991; Yan *et al.,* 2011). This explains the greater body weight gain in piglets fed creep 5 because the provision of dry diets to piglets improves body weight in the post-weaning period (Pluske *et al.,* 2018) through maturation of the digestive process, such as stimulation of digestive enzymes (Pluske, 2016; Jayaraman & Nyachoti, 2017).

The fact that piglets show a reduction in body weight in the nursery phase can be attributed to the stress promoted by weaning and the physical form of the diet, in addition to the social–environmental interactions that affected feed intake (Celi *et al.,* 2017; Moeser *et al.,* 2017; Pluske *et al.,* 2018). It is possible that this dietary transition caused changes in intestinal morphology, triggering impaired performance (Adeowole, 2016; Jayaraman & Nyachoti, 2017). Although the precise mechanisms that favour post-weaning anorexic events are not fully elucidated (Wellock *et al.,* 2013), loss of contact with the mother and litter of origin, structural changes, social and environmental tensions, nutritional transition, and intestinal health are factors that compromise piglet performance post-weaning (Adeowole, 2016; Celi *et al.,* 2017; Jayaraman & Nyachoti, 2017; Liu *et al.,* 2018; Pluske *et al.,* 2018).

The amount of diet provided stimulates the production of digestive enzymes (Pluske *et al.,* 2016) and is important to meet nutritional requirements as piglets grow (Pluske *et al.,* 2018). Piglets exhibit greater feed intake transition at this phase (Bruininx *et al.,* 2002; Dong & Pluske, 2007; Cabrera *et al.,* 2013), favouring the development of the digestive and absorptive structures of the gastrointestinal tract (GIT) (Bruininx *et al.,* 2004; Cabrera *et al.,* 2013). This explains the higher body weight gain in the first week of nursery in piglets fed creep 5.

When evaluated from d5 to d7, the creep 5 piglets showed a higher frequency of udder massaging (P =0.020), standing (P =0.030), and other behaviour (P =0.003), compared to those of creep 10 (Table 2); however, the creep 10 piglets had greater social interaction (P =0.020) and movement (P <0.0001). A difference (P <0.0001) between groups was verified from d8 to d14 of suckling, in which piglets on creep 5 explored the environment and moved more, but lay down less. From d15 to d20, piglets in creep 5 lay near to the sow (P =0.041), explored the environment (P =0.002), explored the feeder (P =0.0008), and sat (P =0.012) more, lay down less in the creep (P <0.0001).

Table 2 Effect of starting creep feeding at different ages on the frequency of behavioural activities in piglets

	d5–d7 (%)[1]			**d8–d14 (%)[1]**			**d15–d20 (%)[1]**		
Parameters	**Creep 5[2]**	**Creep 10[2]**	***P*-value[3]**	**Creep 5**	**Creep 10**	***P*-value**	**Creep 5**	**Creep 10**	***P*-value**
Lying down in the creep	19.55	17.61	0.284	20.52	28.64	*	18.52	29.09	*
Lying down near the sow	26.85	27.17	0.875	25.56	27.93	0.131	27.81	24.19	0.041
Social interaction	2.07	5.16	0.020	3.28	2.61	0.273	2.00	2.74	0.218
Fighting for teats	0.36	0.75	0.240	0.19	0.47	0.141	0.26	0.20	0.749
Udder massaging	11.80	8.68	0.020	9.98	8.39	0.125	10.26	9.01	0.297
Sucking	19.55	20.00	0.808	19.62	18.83	0.573	17.87	18.41	0.726
Exploring the environment	2.25	1.64	0.342	3.18	0.95	*	4.45	2.15	0.002
Exploring the feeder	1.44	1.13	0.558	2.23	2.22	0.973	2.13	0.49	0.0008
Drinking water	0.00	0.00	-	0.05	0.16	0.296	0.26	0.20	0.749
Eating feed	0.00	0.00	-	0.05	0.00	0.438	0.13	0.00	0.250
Exploring the pacifier	0.00	0.00	-	0.10	0.00	0.273	0.00	0.00	-
Urinating	2.52	1.51	0.128	3.04	2.37	0.255	2.32	1.57	0.183
Moving	6.04	12.20	*	7.84	4.35	*	7.03	7.93	0.392
Standing	4.50	2.64	0.030	1.76	1.42	0.455	3.16	2.25	0.201
Defecating	0.81	0.63	0.646	0.81	0.32	0.079	0.39	0.20	0.394
Others	1.62	0.25	0.003	0.86	0.55	0.396	1.55	0.88	0.141
Sitting	0.63	0.63	0.999	0.95	0.79	0.634	1.87	0.69	0.012

[1]d5–d7: Behavioural assessments from days 5 to 7 of suckling; d8–d14: Behavioural assessments from days 8 to 14 of suckling; d15–d20: Behavioural assessment of days 15–20 of suckling. [2]Creep 5 = piglets that received solid feed from 5 days of age; Creep 10 = piglets that received solid feed from 10 days of age. [3]Significance level according to Fisher's test; * = P <0.0001

These behaviours are expected at this phase, as piglets suck approximately every hour and in the interval between suckles, they spend most of their time sleeping (Hötzel, 2007). In the d5–d7 assessments, creep 10 piglets showed greater social interaction because they stayed longer in motion compared to creep 5 piglets, which spent more time standing and udder massaging. Thus, during the first days of age, the piglets depend mainly on the interrelationships between the piglets and the sow to express their investigative behaviour.

From d8–d14, piglets in the creep 10 group stayed longer lying inside the creep. This behavioural posture suggests a lack of knowledge in piglets that received creep 10 in their search for nutrition, and this reflects less accessibility to the feeder, affecting the initial intake behaviour. However, piglets that consumed creep 5 spent longer moving and the greater frequency of exploratory behaviour may be related to the visual, olfactory, and tactile stimulus of the presence of feed in the feeder. It is known that pre-exposure to smell or the presence of new feed can reduce food neophobia (Nicol & Pope, 1994) and this can influence feed intake during the suckling phase (Kuller *et al.,* 2010).

When assessed from days 15–20, piglets in the creep 5 group spent more time exploring the feeder and the environment, lying close to the female, and sitting down. The reason piglets spend more time sitting has been reported as a behavioural indicator of stress in pigs (Hötzel *et al.,* 2007) and serves as a warning that the management performed did not change the behavioural dynamics of the animals. Exploratory behaviour and social interaction activities have the ability to promote feeding behaviour (Pajor *et al.,* 1991) and it may be possible to increase feed intake with a feeder that stimulates exploratory behaviour (Kuller *et al.,* 2010). Therefore, it is also possible to change exploratory behaviour by anticipating the provision of feed to piglets.

Creep feeding can improve performance during the suckling period by supplementing nutritional requirements of piglets, but this depends on the maturation of the GIT and the need for compensatory nutrients (Pajor *et al.,* 1991), as well as the possibility to express feeding behaviour (Wattanakul *et al.,* 2005). In summary, damage to GIT in piglets at weaning can be alleviated by manipulating the development and stability of the intestinal microbiota through the combination of ingredients in the diet (Cabrera *et al.,* 2013) because there is a possible effect of the intestinal microbiota on animal behaviour via the central nervous system (Carabotti *et al.,* 2015).

There were no differences (P >0.05) in the haematological and biochemical parameters of piglets on the different treatments (Table 3).

Table 3 Averages (± SE) of the effect of starting creep feeding at different ages on the haematological and biochemical parameters in piglets

Parameters[1]	**Creep 5**[2]			**Creep 10**[2]			***P*-value**[5]
	N[3]	**Average**	**SE**[4]	**N**	**Average**	**SE**	
13th day of age							
Total leukocytes, $\times 10^3$ µL^{-1}	21	11.44	2.30	15	10.32	2.66	0.610
Haematocrit, %	21	45.72	12.84	15	43.66	8.53	0.489
MCV, fL	21	79.54	9.56	15	76.36	9.61	0.313
MCHC, g dL^{-1}	21	26.60	2.70	15	26.44	2.71	0.652
Platelets, mil µL^{-1}	21	441.38	165.96	15	447.80	252.71	0.853
Amylase U L^{-1}	20	472.10	270.92	13	404.80	230.50	0.386
Weaning (21 days old)							
Total leukocytes, $\times 10^3$ µL^{-1}	21	12.13	2.78	15	14.04	3.90	0.936
Haematocrit, %	21	42.16	8.35	15	42.82	5.11	0.966
MCV, fL	21	69.33	13.02	15	70.62	5.63	0.613
MCHC, g dL^{-1}	21	26.82	1.71	15	26.81	1.79	0.813
Platelets, mil µL^{-1}	21	537.14	233.95	15	534.53	198.69	0.693
Amylase U L^{-1}	19	483.68	194.81	13	446.23	200.87	0.401
First week of nursery (28 days old)							
Total leukocytes, $\times 10^3$ µL^{-1}	18	19.11	7.65	10	19.39	6.38	0.795
Haematocrit, %	18	50.75	7.66	10	57.55	13.73	0.981
MCV, fL	18	72.41	5.45	10	69.69	5.63	0.121
MCHC, g dL^{-1}	18	24.99	1.23	10	25.29	1.16	0.379
Platelets, mil µL^{-1}	18	358.67	194.22	10	312.60	173.89	0.500
Amylase U L^{-1}	17	395.07	227.06	11	421.36	209.21	0.906

[1]MCV = Mean corpuscular volume; MCHC = Mean corpuscular haemoglobin concentration; [2]Creep 5 = piglets that received solid feed from the fifth day of age; Creep 10 = piglets that received solid feed from the tenth day of age. [3]Piglets number. [4]SE = Standard error. [5]Significance level

To our knowledge, there appear to be no reports on haematological parameters and amylase involving piglets at different ages at the start of creep feeding. However, the collection at the 13th day of age showed that 36% of the animals were immunosuppressed and 22% were dehydrated (Thorn *et al.,* 2010). This is possibly due to immunological immaturity and passive defence in piglets, which favours infection by enteric pathogens (Spencer *et al.,* 1989). Furthermore, the amount of milk at the end of suckling is insufficient for piglet growth at this phase (Harrel *et al.,* 1993), which presumably explains 11% of the animals being dehydrated and 27% being immunosuppressed at weaning (Thorn *et al.,* 2010). Another possible factor would be the chronic stress suffered by these animals (Pitts *et al.,* 2000) as a consequence of teat disputes and milk shortage.

In the collection after the first week of nursery, haematocrit results above the reference value were observed in 50% of the animals, in addition to leukocytosis in 21% of the animals, indicating a condition of dehydration, infection, or stress (Randolph *et al.,* 2010). According to Wu *et al.* (2011) and

Xiao *et al.* (2012), this is attributed to low intake and digestive capacity in the first days of the nursery, also to post-weaning diarrhoea caused by exposure to microorganisms, combined with their attempt to adapt to the new environment. However, the variation in haematological parameters can be affected by the sampling time, promoting stress responses with an increase in leukocyte concentration, or general health status, e.g., the effect of disease (Randolph *et al.,* 2010).

Amylase concentration values were lower than the range of 500–600 U L^{-1} reported by Pierzynowska *et al.* (2018). Abrupt weaning or low stimulation of feed intake during suckling can reduce piglet digestive capacity because it is related to reduced enzyme activity (Marion *et al.,* 2003). Considering the absence of specific substrates during suckling, it is possible that the amylase concentration in the blood would be responsible for regulating the carbohydrate uptake (Pierzynowska *et al.,* 2018). However, piglets showed a reduction in amylase concentration in the first week of the nursery and this is attributed to the effects of post-weaning stress, affecting feed intake (Celi *et al.,* 2017; Jayaraman & Nyachoti, 2017; Moeser *et al.,* 2017) and consequently the available amylolytic substrates.

Conclusions

The results of the present study indicate that piglets submitted to creep feeding management at five days of age showed better growth performance post-weaning and in feed intake capacity associated with exploratory behaviour than piglets fed from ten days of age. In addition, creep feeding management did not affect blood haematological parameters and amylase concentration.

Author Contributions

All authors contributed equally and commented on the early and final version of the manuscript.

Conflict of Interest

The authors declared no conflicts of interest.

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