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Performance and feed preference of weaned piglets fed with corn grain silage subjected to different rehydration sources

[*Desempenho e preferência alimentar de leitões desmamados alimentados com silagem de grão de milho submetido a diferentes fontes de reidratação*]

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ABSTRACT

The objective of this study was to evaluate the effects of diets composed of corn grain silage rehydrated with water and acid whey on the productive performance and feed preference of weaned piglets. We evaluated 120 piglets weaned in the pre-initial phase I for productive performance, and diarrhea, depression, and thinness states using the following diets: control ration with dry corn grain (CR); and corn grain silage rehydrated with water (CGSR+W); water and inoculant (CGSR+WI); and acid whey (CGSR+AW). To determine feed preference, 20 weaned piglets received reference (CR) and test (CGSR+AW) diets in two phases, restricted and free-choice. Analysis of variance, chi-square test, and t-test were applied at 5% of significance. There were no differences (p>0.05) in growth performance among piglets fed the four experimental diets. Severe thinness and depression were recorded among piglets fed CGSR+W and CR (p<0.05). In the restricted phase, consumption of CGSR+AW resulted in higher daily feed intake (DFI) (p<0.05), while piglets in the free-choice phase showed a higher DFI and spent more time consuming CR, with a 56.39% preference (p<0.05). Rehydrated corn grain silage promoted feed acceptability and performance of piglets in the first weeks post-weaning.

Keywords: acid whey, diarrhea; intake, preference, silage, weaning

RESUMO

O objetivo do presente estudo foi avaliar os efeitos de dietas compostas de silagem de grão de milho reidratado com água e soro de leite no desempenho produtivo e preferência alimentar de leitões desmamados. Foram avaliados 120 leitões desmamados durante a fase pré-inicial I para o desempenho de crescimento, escores de diarreia, depressão e magreza submetidos às dietas: grão de milho seco (CR); silagem de grão de milho reidratado com água (CGSR+W); água e inoculante (CGSR+WI); soro de leite (CGSR+AW). Para avaliar a preferência alimentar, 20 leitões desmamados receberam dieta referência (CR) e dieta teste (CGSR+AW) em duas fases, restrita e livre escolha. Foi aplicado análise de variância, teste de qui-quadrado e t-test à 5% de significância. Não houve diferenças (p>0,05) no desempenho de crescimento entre os leitões alimentados com as quatro dietas experimentais. Leitões alimentados com CGSR+W e CR foram severamente magros e apresentaram escore de depressão (p<0,05). Na fase restrita o CDR foi maior para CGSR+AW (p<0.05), enquanto na fase de livre escolha os leitões mostraram maior CDR e passaram maior tempo consumindo a CR, com preferência de 56,39% (p<0.05). A CGSR promoveu melhor aceitabilidade e desempenho dos leitões nas primeiras semanas pós-desmame.

Palavra-chave: consumo, desmame, diarreia, preferência, silagem, soro de leite

INTRODUCTION

In intensive swine production systems, early weaning of piglets (between 21 and 28 d of age) is commonly practiced, and corresponds to a critical event particularly in the first two weeks following segregation (Villagómez-Estrada *et al.*, 2020). At this stage, several stress factors, such as milk deprivation, social disruption, unknown environment, and reluctance to a new diet, among others, are associated with a delay in

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starting or determining food consumption below basic needs. Consequently, changes in the intestinal morphology and microbiome of the animals can result in diarrhea and lead to poor growth performance and increased mortality rate (Heo *et al.,* 2013; Oostindjer *et al.,* 2014).

To minimize these effects on the performance of weaned piglets, new feeding strategies, such as providing a diversified meal (regarding flavors, textures, and sensory characteristics), encourage exploratory behavior, and piglets may spend more time in the trough, increasing feed intake (Middelkoop *et al.,* 2019). Feed characteristics, such as flavor, fiber content, a wide variety and textures of cereals, and highly digestible starch (Solà-Oriol et *al.,* 2009; Solà-Oriol et *al.,* 2014), have been investigated to determine the impact of feed taste on weaned piglets.

The process of ensiling reduces non-starch polysaccharides, increases starch availability, produces organic acids during fermentation, and prompts changes regarding odor and texture that can improve the palatability of feed ingredients (Kanengoni *et al.,* 2015).

The ensiling of dry corn grains requires rehydration, which is commonly performed using water (Rezende *et al.,* 2014). The supply of rehydrated corn silage to weaned piglets could play an important role in this critical phase, as the ensiling process likely improves digestibility and promotes the acidification of the corn grain; these effects are reflected in greater animal growth performance (Kanengoni *et al.,* 2015) and improved gut health, with rare occurrences of diarrhea (Suiryanrayana and Ramana, 2015). The advantages of preserving food include improved animal performance (Kanengoni *et al.,* 2015) and feed preference may be an essential factor in facilitating feeding initiation in piglets during the post-weaning phase (Solà-Oriol et *al.,* 2014).

An alternative to the use of water in grain rehydration is acid whey, which contains high a water content (940 g/kg), lactose (44–50g/kg), soluble proteins (6–8g/kg), and minerals (7g/kg) (Rostagno *et al.,* 2017). These features support the rehydration of dry corn grain, and acid whey is an additional source of lactic acid bacteria, promoting the acidity of the environment, and positively affecting the chemical-fermentative aspects of the final silage (Rezende *et al.,* 2014).

Based on these concepts, the inclusion of rehydrated corn silage, with water or acid whey, in the diets of weaned piglets could increase feed consumption, improve digestibility of dietary nutrients, and minimize diarrheal occurrence. Additionally, rehydrated corn silage can improve the organoleptic characteristics of diets, such as texture and flavor, and motivate the exploratory behavior of pigs. The objective of this study was to evaluate the growth performance and preference of weaned piglets fed diets composed of rehydrated corn grain silage, with water or acid whey, compared to a diet containing dry corn grain.

MATERIAL AND METHODS

The experiments were approved by the Committee on Ethics and Use of Animals (CEUA) (registration no. 1738.2019.75). Two experiments were conducted with weaned piglets. Experiment 1 was designed to study the effect of diet on animal performance. Experiment 2 was designed to evaluate the feed preference of weaned piglets for rehydrated grain corn silage.

In Experiment 1, 120 crossbred (Choice genetics) piglets, castrated males and females, with an average age of 21 days, and initial weight of 6.03±0.75 kg were used. The experiment was started at weaning and concluded at 32 days of age, during the pre-initial phase I. The animals were housed at a nursery facility in groups of three per pen. Each pen (3 m^2) had a partially slatted plastic floor, linear feeders, and nipple-type drinkers, and was heated using 200 W infrared (IR) lamps. Animals had *ad libitum* access to water.

The experimental treatments were as follows: control ration (CR, dry grain corn); corn grain silage rehydrated with water (CGSR+W); corn grain silage rehydrated with water combined with inoculant (CGSR+WI); and corn grain silage rehydrated with acid whey (CGSR+AW). The average nursery temperature was 26.81±2.55ºC. The piglets were distributed in a randomized block design according to their weight and sex, with 10 repetitions per treatment. A pen with three animals was used as the experimental unit.

In Experiment 2, 20 piglets (10 castrated males and 10 females; Choice Genetics), with initial average weight of 6.139±1.64 kg and weaned at 21 days of age were used. The animals were randomly distributed into 10 pens according to weight and sex, to form 10 pairs of animals, which were evaluated for 21 days. The stalls had a surface area of 1.5 m^2, with a fully slatted plastic floor and a nipple-type drinker. The piglets were adapted to the pens for 7 d before the experiment began; thus, at the beginning of Experiment 2, the animals were 28 days of age. Four round plate-type feeders were installed in each stall to reduce competition for feeding places and provide equally the two diet options, CR and CGSR+AW. Trough content was switched during the experiment to eliminate the possibility of preference for trough position (left or right side). A thermometer (TR10, Western®, China) was fixed at the center of the shed to monitor room temperature.

The experimental diets (Table 1) that contained rehydrated corn grain silages were formulated to meet the same nutritional characteristics of CR, which was formulated with dry ingredients. The dry matter (DM) of corn grain (880 g/kg DM) used in the preparation of the CR ration represents the reference value. The DM values of the silages were determined and the rations of the respective diets were adjusted within the level of corn DM in CR. The digestible energy values of rehydrated corn grain silages were based on the results obtained by Silva et al. (2006), which are used for the formulation of experimental diets to meet the minimum nutritional requirements of piglets with high genetic potential at the age of 21–32 days (pre-initial phase I). These values are based on the nutritional recommendations of the Brazilian Tables for Poultry and Swine (Rostagno *et al.,* 2017).

The dry corn grains used for silage production were processed with an average particle size of 1.5 mm and rehydrated (with water or whey) to attain a moisture level of 370 g/kg. The ensiled mass was placed in drum-type silos with a capacity of 250 L and stored for 90 days until opening. The piglets were fed total mixed ration (TMR) provided *ad libitum* according to each treatment; TMR composed of dry corn, CGSR+W, or CGSR+AW, soybean meal, and other ingredients (Table 1). The diets containing rehydrated corn grain silage were prepared daily in a "Y" type mixer for 5 min by mixing the ensiled mass portion and the other ingredients of the total ration (Table 1).

Table 1. Ingredients composition and calculated nutritional values of the experimental diet for weaned piglets

Ingredients (%)	Pre-initial phase I (21 to 32 days)
Dry corn grain (8,7% CP)	57.0120
Soybean meal (47% CP)	11.1522
Whey powder	14.0000
Star pro 25	8.0000
Soy protein concentrate	1.1045
Spray dried blood plasma	5.0000
Limestone	0.2963
Dicalcium phosphate	1.3437
L-lisine hydrochloride	0.6628
Zinc oxide	0.3000
L-threonine, 98%	0.2271
DL-methionine	0.3747
L-tryptophan	0.0578
Antioxidant (Banox)	0.0100
Mycotoxin adsorber	0.1500
Salt	0.0587
Vitamin premix[1]	0.1500
Mineral premix[2]	0.1000
Total	100.00
Nutritional levels	
Protein (%)	19.00
Fat (%)	4.59
Calcium (%)	0.91
Available phosphorus (%)	0.46
Metabolizable energy (Kcal/kg)	3403
Digestible lysine (%)	1.45
Total lactose (%)	9.85
Digestible methionine + cystine (%)	0.81
Digestible threonine (%)	0.97
Digestible tryptophan (%)	0.28
Mineral matter (%)	6.45

[1]Levels per kg of Vitamin Premix: vitamin A (min) 6.000 UI; vitamin D3 (min) 1.500 UI; vitamin E (min) 15.000mg; vitamin K3 (min) 1.500mg; vitamin B1 (min) 1,350mg; vitamin B2 4.000mg; vitamin B6 2,000mg; vitamin B12 (min) 20mg; niacin (min) 20.000mg; pantothenic acid (min) 9,350mg; folic acid (min) 600mg; biotin (min) 80mg; selenium (min) 300mg.
[2]Levels per kg of the mineral premix: iron (min) 100mg; copper (min) 10mg; manganese (min) 40 g; cobalt (min) 1.000mg; zinc (min) 100mg; iodine (min) 1.500mg.

After opening the silos, silage (Table 2) and TMR samples, which formed the experimental diets (Table 3), were evaluated for chemical composition (AOAC, 2000). Neutral detergent fiber (NDF) was assayed using heat-stable alpha amylase and sodium sulfite (aNDF); acid detergent fiber (ADF) and lignin contents were evaluated using sulfuric acid and corrected for ash (Van Soest *et al.*, 1991). To evaluate the fermentative profile of silage and TMR samples, the buffer capacity (BC) and ammonia nitrogen ($N\text{-}NH_3$) content were determined according to the method described by Playne and McDonald (1966), and the values of hydrogen ionic potential (pH) was measured using a potentiometer (AZ Temp Meter; AZ Instrument Corp., Taichung City, Taiwan) at the time of opening the silos and during the experimental period (AOAC, 2000). The chemical-fermentative composition of the silage samples was determined using a near-infrared spectrometer (NIRS™ DS2500; Foss, Hillerød, Denmark) (Table 4) at the 3rlab® laboratory (Chapecó, Santa Catarina, Brazil).

Table 2. Chemical composition (mean ± SE) of silages (g/kg of DM) fed to the weaned piglets

	Pre-initial phase I (21 to 32 d)		
Variables[1]	CGSR+W	CGSR+WI	CGSR+AW*
DM	663.1±0.06	677.3±0.78	709.9±0.02
OM	989.6±0.03	987.8±0.12	980.7±0.48
Ash	10.4±0.03	12.2±0.12	19.3±0.48
CP	99.0±0.14	104.7±0.13	102.3±0.27
EE	34.5±0.12	40.9±0.10	30.5±0.18
NDF	89.4±1.42	91.7±0.83	94.6±0.27
ADF	17.6±0.12	21.9±0.20	21.2±0.07
Lig	01.4±0.01	02.2±0.01	09.4±0.01
TCHO	856.1±0.16	842.3±0.02	847.9±0.73
TDN	766.7±1.49	750.5±0.81	753.3±0.73
$N\text{-}NH_3$	00.5±0.001	00.4±0.005	00.3±0.004
BC	15.10	22.54	24.04
pH	4.14	3.89	4.17

[1]DM, Dry matter (% of natural matter); OM, Organic matter; Ash, Mineral matter; CP, Crude protein; EE, Ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; Lig, lignin; TCHO, Total carbohydrates; TDN, Total digestible nutrients; N-NH3, ammoniacal nitrogen (g/kg of total nitrogen); BC, Buffering Capacity (e.mg/100g DM); CGSR+W, corn grain silage rehydrated with water; CGSR+WI, corn grain silage rehydrated with water combined with inoculant; CGSR+AW, corn grain silage rehydrated with acid whey. *The acid whey presented following composition: 64.4g/kg of DM, 9.3g/kg of CP, 4.1g/kg of ash, 2 g/kg of fat, pH was 4.84, and the pH of lactic acid was 0.34 of according AOAC (2000). The dry corn grain used for silages production was analyzed (AOAC, 2000), with the following composition: 880g/kg of DM, 856g/kg of CP, 12g/kg of ash, 120g of NDF, 25g/kg of ADF, 31g/kg of EE, 863.5g/kg of TCHO, and TDN of 730. 3g/kg.

Table 3. Chemical composition (mean ± SE) of the experimental diets fed to the weaned piglets

	Diets			
	CR[1]	CGSR+W	CGSR + WI	CGSR +AW[2]
Nutrients (g/kg DM)	Pre-initial phase I (21 to 32 days)			
DM	919.9±0.11	743.4±0.13	749.4±0.08	757.6±0.08
OM	945.9±0.10	953.9±0.03	953.1±0.02	956.7±0.16
Ash	54.1±0.10	46.1±0.03	46.9±0.02	43.3±0.16
CP	169.7±0.59	204.9±1.69	193.7±0.10	185.2±0.25
EE	54.3±1.96	36.3±0.003	43.4±0.21	36.2±0.05
NDF	76.2±0.75	84.4±0.59	62.5±0.42	85.9±0.14
ADF	16.4±0.20	18.7±0.09	16.1±0.25	19.6±0.03
Lignin	02.0±0.14	02.4±0.08	02.0±0.03	03.9±0.22
TCHO	755.2±0.50	712.6±1.72	731.6±1.23	735.3±0.39
TDN	649.9±0.71	696.7±1.49	669.0±3.03	649.4±0.50
$N\text{-}NH_3$ (g/kg total nitrogen)	00.2±0.001	00.3±0.001	00.3±0.002	00.3±0.001
BC (e.mg/100g DM)	21.12	23.52	24.18	24.15
pH	6.82	5.37	4.98	5.36

DM, Dry matter (% of natural matter); OM, Organic matter; Ash, Mineral matter; CP, Crude protein; CE, Crude energy; EE, Ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; TCHO, Total carbohydrates; TDN, Total digestible nutrients; N-NH3, ammoniacal nitrogen (g/kg of total nitrogen); BC, Buffering Capacity (e.mg/100g DM); CR, control ration; CGSR+W, corn grain silage rehydrated with water; CGSR+WI, corn grain silage rehydrated with water combined with inoculant; CGSR+AW, corn grain silage rehydrated with acid whey. [1]Reference test = dry corn grain (CR); [2]Test diet = corn grain silage rehydrated with acid whey (CGSR +AW).

Table 4. Chemical-fermentative quality of silages determined using the NIRS system

Variables[1] (g/kg DM)	CGSR+W	CGSR+WI	CGSR+AW
DM (g/kg of NM)	683.3	673.1	664.9
Moisture	316.7	326.9	335.1
CP	91.6	92.3	92.9
Protein soluble g/kg CP	466.8	467.9	443.8
Protein available	90.8	91.7	92.1
ADIP	00.8	00.7	00.8
NDIP	03.0	02.7	02.5
ADIP g/kg CP	08.5	07.2	08.8
ADF	29.8	29.4	29.7
NDF	87.9	82.3	79.6
aNDFmo	81.5	77.2	73.2
Lignin	05.0	04.9	05.0
Lignin g/kg NDF	56.7	60.0	62.4
Sugars (Carbohydrates soluble in water)	44.1	49.5	48.4
Starch	710.0	705.7	704.2
Starch g/kg NFC	917.3	909.3	902.7
Lipids	32.2	34.9	32.8
Ash	17.4	17.2	17.0
Calcium	00.5	00.5	00.5
Phosphorus	02.1	02.1	02.0
Potassium	03.8	03.9	03.7
Magnesium	00.9	00.9	00.8
Sulfur	00.8	00.9	00.9
Lactic acid	22.3	25.3	20.6
Acetic acid	01.6	02.1	01.4
Protein equivalent of $N\text{-}NH_3$	02.3	02.4	02.5
$N\text{-}NH_3$ (g/kg da CP)	24.8	25.6	26.9
pH	4.42	3.77	4.33
Kd of starch (using 3.7 h) %h	15.44	15.01	14.46

[1] Variables: DM, Dry matter (% of natural matter); CP, crude protein; ADIP, acid detergent insoluble protein; NDIP, Neutral detergent insoluble protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; aNDFmo, neutral detergent fiber with amylase and expressed excluding residual ash; N-NH3, ammoniacal nitrogen. Kd, Fractional rate of degradation. Analyses determined by the laboratory 3rLab

To evaluate growth performance, the piglets were individually weighed (Mettler Toledo 9091 scale; Mettler-Toledo Inc.; Columbus, Ohio, United States) at the beginning of the experiment and at the end of each phase. Feed consumption was calculated during constant time intervals, as the total supplied minus trough leftovers and waste. The daily weight gain (DWG), daily feed intake (DFI), and feed conversion (FC) were evaluated at the end of each phase, considering the total experimental period. Data on diarrhea, and depression and thinness states were recorded daily by a single trained observer using direct observation at 5 p.m. during the experimental period. The number of medicated animals per treatment was also recorded during the experiment.

Assessment of diarrhea was performed according to the method described by Sobestiansky and Barcellos (2007), using the following classification of stool consistency: 0, normal consistency; 1, semi-solid; 2, sticky; and 3, aqueous. The number of diarrheal occurrences with scores of 2 and 3, and the total number of diarrheal occurrences were evaluated per treatment and the diarrhea incidence and severity were calculated using the following equations:

$$\text{Diarrhea incidence} = \frac{\text{number of animals with diarrhea score} \geq 2}{\text{number of animals per treatment}}$$

$$\text{Diarrhea severity} = \frac{\text{sum of diarrhea scores}}{\text{total number of animals evaluated in days}}$$

The depression status was assessed based on the clinical signs exhibited by the piglets and classified as follows: 0, vivacious, alert, and responsive animal; 1, standing and isolated, but quickly responding to the stimulus; 2, upright and isolated, with a lowered head posture, and may exhibit muscle weakness and delayed response to the stimulus; and 3, depressed, lying down, and reluctant to stand up (Rossi *et al.*, 2021). Thinness was evaluated based on observation of the animals and scored as follows: 0, normal abdomen, full, and round flanks; 1, small bowel and flat flanks; 2, severely thin and empty flanks (Spiehs *et al.,* 2008). The thinness and depression states reflect the number of recorded incidents per treatment.

Piglet feed preference was assessed during two phases. The first was a 10-day restricted phase (Fraser and Matthews, 1997), whereby CR and CGSR+AW were each supplied for 5 days (that is, CR was supplied during the first 5 days and CGSR+AW the last 5 days). In the second phase, animal preference was assessed through free choice for feed type, according to the methodology proposed by Fraser and Matthews (1997). During this stage, both diets were provided for six days and the piglets' behaviors were monitored.

The TMR of each diet (Table 1) was provided *ad libitum* according to the requirements of each experimental phase. The TMR was weighed immediately after preparation and supplied twice daily, at 8 a.m. and 4 p.m. DFI was obtained by recording the total feed provided minus leftover feed.

To determine preference during the free-choice phase, the relationship between the reference and test diet was calculated using the following equation (Solà-Oriol *et al.* 2009):

$$\% \text{ preference} = \frac{\text{Test diet intake}}{(\text{Test diet intake}) + (\text{Reference diet intake})} \times 100$$

where the reference diet refers to CR and the test diet to CGSR+AW.

Animal behavior during the feed preference test was recorded by a single observer using direct observation and instantaneous scan sampling at 5-min intervals (Altmann, 1974) for two 2-h sessions and using pen and paper for annotation. Two animals from the experimental group (pairs) were identified with a marker stick. The animals were monitored individually for 16 consecutive days from 8 to 10 a.m. and 4 to 6 p.m. (i.e., for 2 h after feed provision) (Oliveira *et al.,* 2018). Thus, a total of 48 scans per piglet were recorded daily. In each scan, the following behaviors were recorded and their duration was expressed in min (Martin and Bateson, 1993): eating (animal with head over or within the feeder, in the process of chewing, and with feed in the mouth); animal position (lying, sitting, or standing); resting (lying down); drinking water (animal in the trough drinking water); and number of visits to the trough (approaching the trough without feed intake). At the end of the test period, the animals were individually weighed (Mettler Toledo 9091 scale; Mettler-Toledo Inc.; Columbus, Ohio, United States).

The performance results of Experiment 1 were subjected to analysis of variance (ANOVA), and the means were compared using the Tukey's test and Kruskal–Wallis nonparametric test at 5% significance, using the statistical software RStudio (v. 3.6.0; 2019) with the dbc ExpDes.pt package. The normality of the residues was assessed using the Shapiro–Wilk test and the homogeneity of variance was assessed using the Anscombe–Tukey test with P <0.05. Data on diarrhea, thinness, depression, and number of medicated animals were compared using the Chi-square test at 5% significance. For the performance data, the following mathematical model was applied:

$$Yij = \mu + ti + bj + eij$$

where,
Yij = observed response to treatment (i) in block (j);
μ = overall mean;
bj = effect blocks of each repetition;
ti = treatment effect; and
eij = residual error effect.

In Experiment 2, the data of the 48 scans per piglet (n=20) per day were calculated as the total duration of each behavior in minutes. These observations of each behavior per piglet per day and in each phase (5 d in restricted diet and 6 d in the free-choice phases) were averaged to form a mean value of each behavior per piglet pair per day. In this way, the final value of duration of each behavior per d was calculated for each pair of weaned piglets (10 pairs in total).

The means were compared using ANOVA and data normality was verified using the Shapiro–Wilk test in software R (version 3.6.0; 2019) at the significance level of α=0.05. For the analyses of %preference, the mean DFI per pair in each diet during the free-choice phase was compared with a neutral value of 50% using the Student's t-test in R. Data are presented as the mean ± standard error.

RESULTS

There were no differences (*P*>0.05) in growth performance among piglets fed the four experimental diets (CR, CGSR+W, CGSR+WI, and CGSR+AW) (Table 5). The diarrhea scores did not differ among the different experimental diets (*P*>0.05). However, we observed a trend (*P*=0.060) in the number of occurrences and severity of diarrhea (score 3) in the CGSR+W and CGSR+WI groups (Table 6) with 6 and 7 occurrences, respectively. In the CR and CGSR+AW groups, 10 and 17 occurrences were observed, respectively. Regarding the medication of animals, there was no difference among different diets, regardless of the disease diagnosed (*P*>0.05).

The thinness scores (Table 6) were different among experimental diets (*P* <0.05). The CR resulted in a lower occurrence of a score of 1 and CGSR+WI in a higher occurrence compared to that in other diets. Severe thinness (score 2) was only observed in the CR and CGSR+W groups (*P*=0.004). The effect of diet on depression severity (score 3) was observed, with higher occurrence in the CR and CGSR+W groups compared to the other groups (*P*=0.000).

Table 5. Performance of piglets weaned fed with diets with the inclusion of corn grain silage rehydrated

Variables[1]	Treatments				P-value
	CR	CGSR+W	CGSR+WI	CGSR+AW	
Pre-initial phase I (21 to 32 d)					
IW (kg)	6.04±0.76	6.04±0.77	6.02± 0.76	6.03±0.75	0.9995
DFI (g/d DM)	231.90±0.06	279.26±0.07	234.37±0.05	240.30±0.05	0.2746
DWG (g/d)	175.61±0.07	210.09 ±0.07	160.02±0.05	174.25±0.03	0.3002
FC	1.42±0.38	1.36±0.13	1.51±0.17	1.38±0.06	0.3896
WF (kg)	7.97±0.95	8.35± 1.47	7.78±1.11	7.95 ±0.90	0.8465

[1] IW, Initial weight (kg); DFI, daily feed intake; DWG, daily weight gain; FC, feed conversion; WF, weight final. CR, control ration; CGSR+W, corn grain silage rehydrated with water; CGSR+WI, corn grain silage rehydrated with water combined with inoculant; CGSR+AW, corn grain silage rehydrated with acid whey.

Table 6. Number of occurrences and score of diarrhea, the rate of incidence, and severity of diarrhea; number of animals medicated with injectable products, according to the diagnosed diseases; number of occurrences of thinness score and depression score in the total evaluation period

Parameters[1]	Treatments[2]				P-value
Diarrhea score (n)	CR	CGSR+W	CGSR+WI	CGSR+AW	
2	33	30	42	40	0.445
3	10	6	7	17	0.060
Total	43	36	49	57	0.160
Incidence	1.43	1.20	1.63	1.90	-
Severity	0.33	0.20	0.23	0.57	-
Medications (n)					
Pneumonia (cough)	2	2	1	2	0.934
Arthritis	2	3	2	0	0.283
Diarrhea	1	0	1	3	0.438
Thinness score (n)					
0	135	125	117	132	0.679
1	28b	39ab	53a	38ab	0.045
2	7a	6a	0b	0b	0.004
Depression score (n)[3]					
0	148	158	163	159	0.855
1	10	9	6	10	0.746
2	3	0	1	1	0.284
3	9a	2b	0b	0b	0.000

[1]Diarrhea score: 2, sticky; 3, aqueous; Thinness score: 0, normal abdomen, full, and round flanks; 1, small bowel and flat flanks; 2, severely thin and empty flanks. Depression score: 0, vivacious, alert, and responsive; 1, standing and isolated, but quickly responding to the stimulus; 2, upright and isolated, with low head and may exhibit muscle weakness and delayed response to the stimulus; 3, depressed, lying down, and reluctant to stand up. (n): number of occurrences for each score

[2]CR, control ration; CGSR+W, corn grain silage rehydrated with water; CGSR+WI, corn grain silage rehydrated with water combined with inoculant; CGSR+AW, corn grain silage rehydrated with acid whey. [a,b] Different mean in the lines indicate difference by the Chi-square test applied at a significance level of 5%.

The behavioral variables of lying down, standing, eating time, and DFI did not differ ($P<0.05$) between diets during the restricted phase, in which only one diet was provided. A higher DFI value was observed for the test diet (CGSR+AW), with 649.1±152.7 g of DM compared with 509.7±129.4 g of DM in the reference diet (CR) ($P=0.0086$) (Fig. 1a). The longer amount of time spent lying down was observed in the CR group with 276.7±48.3 min/d compared with 237.9±44.9 min/d in the CGSR+AW group ($P=0.0463$) (Fig. 1b). Piglets on CGSR+AW diet exhibited longer standing behaviors compared with those on CR (74.4±28.1 vs 33.2±23.8 min/d, respectively; $P=0.0001$) (Fig. 1c).

Feeding time, drinking water, sitting, and visit to the trough were not different ($P>0.05$) during the restricted phase. The weaned piglets spent 158.9±43.5 min/d feeding on CR and 162.7±38.4 min/d on CGSR+AW ($P=0.6445$). The CR group spent 7.2±6.3 min/d drinking water and the CGSR+AW group, 5.7±6.4 ($P=0.2408$). Weaned piglets on CR and CGSR+AW spent 0.6±1.6 and 0.7±2.0 min/d, respectively, time sitting ($P=0.7866$). Visit to the trough was observed for a duration of 0.7±2.3 min/d in both diet treatments ($P=1.000$).

In the free-choice experiment, the feeding time and DFI differed ($P<0.05$) between diets. The weaned piglets spent more time consuming CR than CGSR+AW (118.7±60.3 vs. 57.3±37.5 min/d, respectively; $P=0.0003$) (Fig. 2a). CR resulted in a higher DFI than CGSR+AW (761.3±178.1 vs. 592.1±207.4 g of DM, respectively; $P=0.0119$) (Fig. 2b). The preference for CGSR+AW was lower ($P<0.05$) than the neutral value of 50% (Fig. 2) and piglets preferred to consume CR (56.39%) over CGSR+AW ($P=0.0041$) (Fig. 2c).

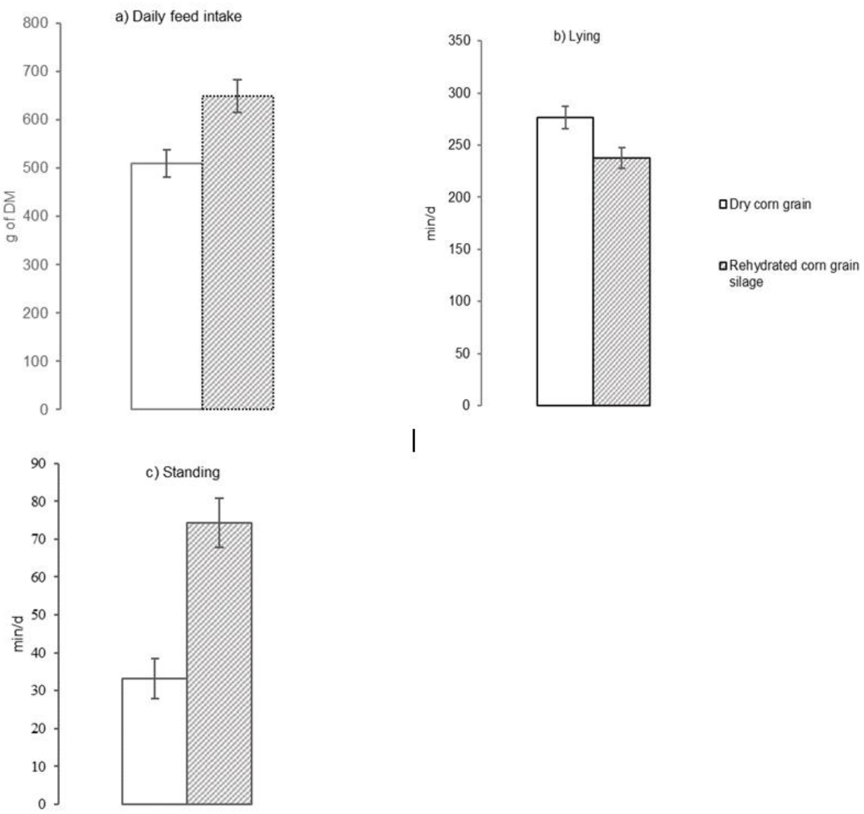

Figure 1. Results from the restricted phase of the experiment. Mean (±SD) daily feed intake (g of DM) (A), and time that weaned piglets (n=20) (min/d) spent lying (B) in the standing (C) of the 2 diets CR = dry corn grain or CGSR+AW = rehydrated corn grain silage ($P<0.05$ for all 3 comparisons).

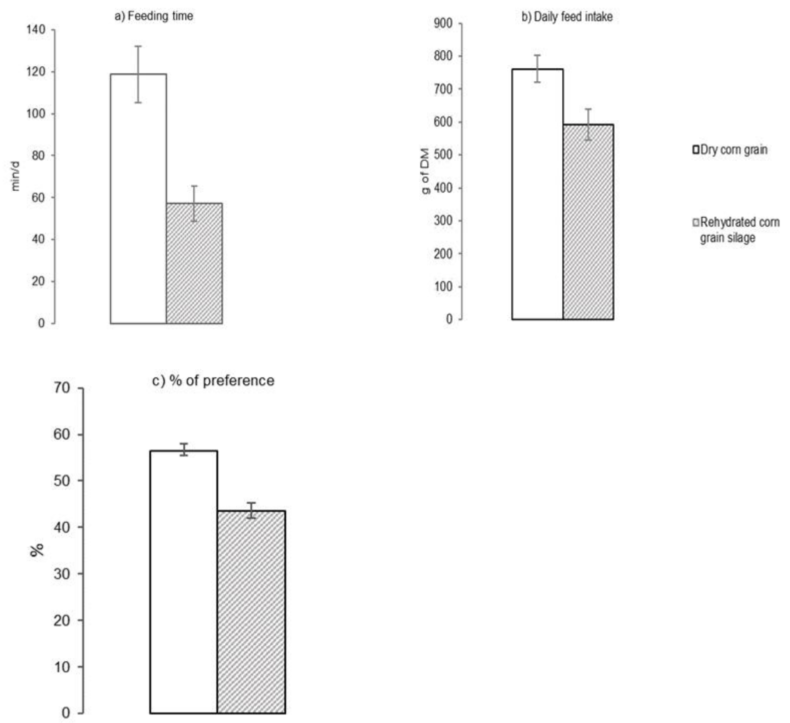

Figure 2. Results from the free-choice phase of the experiment. Mean (±SD) that weaned piglets (n=20) spent feeding time (min/d) (A), daily feed intake (g of DM) (B) and % of preference (C) of the 2 diets CR = dry corn grain or CGSR+AW = rehydrated corn grain silage (*P*<0.05 for all 3 comparisons).

During the free-choice phase, weaned piglets spent an average time of 239.4±61.4 min/d lying down and 57.9±29.1 min/d standing. The recorded duration of drinking water was 8.66±8.6 min/d; sitting, 1.25±2.7 min/d; and visit to the trough, 0.16±0.9 min/d.

DISCUSSION

In pre-initial phase I, the observed DWG values (160.02–210.09 g/d), regardless of the diet provided, were in accordance with those reported previously in the literature (Villagómez-Estrada *et al.*, 2020; Middelkoop *et al.*, 2019) for piglets aged 21–32d (Table 5). However, there were no differences in the parameters of performance for the DWG, FC, and WF. The rations in pre-initial phase I contained high levels of dairy products (98.5g/kg lactose) and spray dried plasma (50g/kg). It is well-known that these attributes favor digestibility, increase consumption, and improve immune status (Dalto *et al.*, 2013). Although these outcomes may have partly masked the expected benefits of diets based on rehydrated corn silage, they possibly justify the lack of advantages of treatments formulated using CGSR, despite the increased starch availability and acid production, and improvement of organoleptic characteristics of silage (Kanengoni *et al.*, 2015; Kung Júnior *et al.*, 2018). The DWG of piglets fed rations containing silage did not differ (*P*>0.05) compared to that of CR-fed animals, that is, the resources used to rehydrate the corn grain (water, acid whey, and inoculant water) were not sufficient to improve growth performance.

Diets formulated with CGSR+W and/or CGSR+WI may have contributed to a lower number of occurrences and severity of diarrhea compared to other diets (Table 6). We observed 6 occurrences of severe diarrhea (score 3) in the CGSR+W group and 7 in CGSR+WI, while the corresponding numbers were 10 and 17 in the CR and CGSR+AW groups, respectively. Diarrhea occurrence, recorded through daily evaluation per pen and treatment, often regarded the same animals that presented with diarrhea for consecutive days; that is, the number of occurrences in the present study is related to the time that the disease affected the piglets. This indicates that the overall number of piglets with diarrhea was low compared to the 120 animals used in the experiment and 28 days of evaluation. This result is further reflected in Table 6 as a low evolution of diarrhea score from 2 to 3.

The diarrhea score has been reduced for all treatments that received the inclusion of silage in the diet, because, due to the fermentation products generated (organic acids), inherent to the ensiling process, they can improve food preservation, pH stabilization, reduction of BC, and feed acidification (Kung Júnior *et al.*, 2018). However, the CGSR+AW did not show this result, leading to the hypothesis that the whey has a high and variable sodium chloride content (not evaluated in this study) (El-Desoki, 2009), which could favor osmotic diarrhea (Guzman-Pino *et al.,* 2015).

These parameters supra cited were identified based on BC and acid pH values of diets formulated with rehydrated corn grain silage (CGSR+W, CGSR+AW, and CGSR+WI) (Table 3) compared to feed based on dry corn grain, which is lower acidified favoring the positive action in and minimizes the incidence and severity of diarrhea (Suiryanrayna and Ramana, 2015). CGSR+W had a pH value of 5.37 while that of CR was 6.82. Among all treatments, the lowest pH value (4.98, Table 3) was documented for CGSR+WI, which comprised inoculated silage. Increased acidity pH in feed consumed by piglets can exert positive effects on digestion, since a reduction in gastric pH can improve the digestion of nutrients and alter the microbial populations in the stomach and other parts of the gastrointestinal tract (Suiryanrayna and Ramana, 2015).

At the same time, it is known that at the commercial weaning age, due to changes in diet and the immaturity of the digestive system, HCl secretion is reduced, which compromises stomach acidification (Aumaitre *et al*., 1995). Therefore, maintaining a low gastric pH by offering feed with rehydrated corn grain silage optimizes nutrient digestion and prevents pathogen overgrowth (Heo *et al.,* 2013). The addition of organic acids to fresh cereals and feed has been used to improve gastrointestinal tract health and minimize the incidence of diarrhea, with a consequent improvement in the performance of post-weaning piglets (Suiryanrayna and Ramana, 2015). In the present study, a positive relationship between organic acids in rations and low incidence of diarrhea in weaned piglets, especially for CGSR+W, CGSR+ WI, can be deduced, since silage contains organic acids (Table 4) that likely contribute to intestinal health. Again, it is worth noting that the CGSR+ AW diet also had a lower pH compared to the control group, but it is presumed that the high and variable concentration of sodium chloride present in whey can impair the diarrhea occurrence diarrhea (Guzman-Pino *et al.,* 2015), when compared to the other treatments that used also rehydrated corn silage.

In addition, the solubilization of fibers (non-starch polysaccharides such as starch) through the ensiling process (Kanengoni *et al.,* 2015; Kung Júnior *et al.,* 2018), may have also contributed to the reduction in diarrheal cases (Heo *et al.,* 2013; Long *et al*., 2018). The insoluble fiber, which is present at greater percentages in the corn grain (Kung Júnior *et al.,* 2018), is weakened, and it may have been more readily available for digestion.

The thinness and depression scores observed in this study are related to the good health of the piglets during the entire experimental period, this is reflected in the prevalence of a score of 0 for these parameters (Tab 6). Notably, these parameters represent a measurement of the quality of the diet, health conditions, and the environment in which the piglets were inserted. The depression and thinness scores were noted daily by pen and treatment, and the number of occurrences of each condition is related to the time the same piglets were affected by a given disease. Regarding animal medication (Table 6), only few animals were treated (for pneumonia [cough], arthritis, and diarrhea), contributing to the high occurrence of a score of 0.

A thinness score of 1, which characterizes piglets with small intestines and flat flanks, was obtained for the CGSR+WI group and may be related to the relatively low DFI and DWG of the piglets in this group in pre-initial phase I (Table 5). Furthermore, this result is directly related to the sick piglets; that is, a score of 1 was recorded for the whole period that the animal did not show improvement and weight gain. The lowest number of occurrences of a thinness score of 1 was recorded in the CR group, which exhibited the second highest DWG among the treatments (Table 5). The CGSR+W and CGSR+AW groups exhibited a low number of occurrences of score 1, due to the highest DFI and DWG of piglets.

Severe thinness (score 2) was observed in the CR and CGSR+W groups and is related to the specific cases of piglets that were clinically affected by arthritis, pneumonia, and diarrhea (Table 6). These conditions required extensive medication and animals remained ill for several days during the assessment period, resulting in compromised weight gain. Overall, the few observed critical cases of thinness (score 2) indicate a satisfactory effect of all rations on consumption in the first few days after weaning. This was particularly evident in the CGSR+WI and CGSR+AW groups, which did not exhibit severe leanness (P<0.05).

Regarding the depression scores, in general, a score of 0 prevailed (Table 6), which indicates that most of the animals, regardless of treatment, were not in a depressed state. The highest incidence of severe depression (score 3) was observed in the CR group (P<0.05). Piglets that presented signs of depression spent considerable time lying down and were reluctant to stand up due to arthritis, pneumonia, and diarrhea. Thus, a high depression score relates to specifically diagnosed and medicated cases. In addition, severe depression refers to the same animals on consecutive days of observation, similar to the recordings of a thinness score of 2.

A depression score of 3 is related to the general health condition of the animal, which may show restricted development during the pre-initial phase. Post-weaning piglets can often exhibit clinical symptoms of arthritis, among other complications, due to procedures such as the cutting or wearing teeth, tail sectioning, and surgical castration (Nannoni *et al.*, 2014). These procedures favor the entry of bacteria and increase the incidence of arthritis, thereby leading to self-directed behaviors and signs of pain, such as prostration, prolonged lying down behavior, and isolation from other animals in the pen. Consequently, loss of productive performance, such as decreased weight gain, which can last up to 70 days after caudectomy (Nannoni *et al.*, 2014; Zoric *et al.*, 2016), has been frequently reported.

The highest DFI was observed in the test diet group (CGSR+AW) in the restricted phase, which highlights the greater acceptability of silage in the diet during the first weeks post-weaning compared with other diets (Fig. 1a). According to Solà-Oriol *et al.* (2009), diets formulated with high-palatable ingredients can contribute to post-weaning diet adaptation and facilitate feed initiation. This may explain the high consumption of CGSR+AW during the restricted phase in the feed preference test, since the piglets had recently been weaned and were still looking for a moist diet similar to that of sow's milk. A diversified diet (regarding flavors, textures, and sensory characteristics) may encourage exploratory behavior. While piglets perform active behavior, more than half of the time is spent rooting through the trough and ground to satiate their natural rooting behavior. Therefore, when the diet is diversified, piglets spend more time performing this behavior in the trough with consequent increases in feed intake (Middelkoop *et al.*, 2019).

The higher consumption of CGSR+AW than other diets during the restricted phase may be related to the greater availability of starch (704.2 g/kg, Table 4) after corn grain silage. Piglets prefer ingredients with high levels of digestible starch and a high glycemic index (Solà-Oriol *et al.*, 2009; Solà-Oriol *et al.*, 2014). Despite a mash-like appearance and granulometry of 1.5 mm (similar to the dry milled corn grain), changes in the organoleptic characteristics of the silage, such as odor, flavor, and texture (Kung Júnior *et al.*, 2018), may have contributed towards stimulating exploratory behavior and, therefore, increased feed intake. Another fact to be considered is that the weakening of non-starch polysaccharides through the silage process (Kanengoni *et al.*, 2015; Kung Júnior *et al.*, 2018) may have contributed to an increase in the passage rate, encouraging piglets to forage more frequently (Middelkoop *et al.*, 2019) and contributing to food initiation in the first week post-weaning (Fig. 1).

The amount of time spent lying down is an indicator of animal welfare, given that changes in this behavior will be prompted in case of discomfort (Fraser and Matthews, 1997). Newly weaned piglets naturally exhibit resting behavior for approximately 18 hours a per day and the duration of this behavior can change due to common stresses prompted by early weaning (at 21 days of age) (Hessel *et al.,* 2006). During the restricted phase, in which the animals received only one of the diets, the time the piglets spent lying down was higher in the CR group (*P*=0.0463) (Fig. 1b), whereas the time spent standing was greater in the CGSR+AW group (*P*=0.0000) (Fig. 1c). These behavioral patterns may be associated with the lower glycemic index of the CR than CGSR+AW diet, contributing to a prolonged period of satiety, since lying down is related to the satiety that a given feed can offer. It appears that an increase in satiety causes the organism to enter a balance state of decreased physical activity and alertness levels to maintain resting behavior (De Leeuw *et al.,* 2008).

The time spent to drinking water in the CGSR+AW group (7.2 min/d) during the restricted phase can be partially explained by the characteristics of the diet, which had a high moisture content. In the CR group, high water intake may be related to feed consumption, given that the CR diet comprised high DM content, which favors frequent searches for water. In general, dry rations, as opposed to wet rations, favor water consumption. Da Silva *et al.* (2002) found the same trend in a group of weaned piglets that received dry feed; the piglets displayed a higher water consumption compared to that of a group that received wet feed. The authors concluded that dry rations have a positive effect on water consumption in the first weeks after weaning. However, the moisture content of CGSR+AW may have facilitated feed intake in the first weeks after weaning, due to the increased interest of the animals in the feed, without compromising the water intake. The exploratory behavior of the piglets, evaluated by frequent visits to the trough, may explain the comparable values for this variable in both diets. Exploratory behavior is common in pigs, particularly in newly weaned piglets, who use curiosity as a way of adapting to the environment, diet, and other individuals (Middelkoop *et al.,* 2019).

Preference tests offer animals a choice between two or more options, the preferred option is selected most frequently, receives the most attention during the available time, or presents the greatest consumption (Fraser and Matthews, 1997). During the free-choice phase (Fig. 2b), consumption of CR resulted in a higher DFI compared with that of CGSR+AW (*P*=0.0119), which may explain why piglets spent more time consuming CR (Fig. 2a) (*P*=0.0003).

The time the piglets spent eating CGSR+AW during the free-choice phase may indicate a low preference for the test diet. According to Fraser and Matthews (1997), offering the animal a choice, regarding diet or environment, is an appropriate way to determine preference as the option that pleases the animal the most. During the restricted phase, the higher consumption of CGSR+AW (*P*<0.05) and feeding time compared with the CR values may be related to the difficulty of the piglets to adapt to dry feed during weaning. According to Bruininx et al. (2004), newly weaned piglets can take up to 36 h to begin feeding. When piglets are exposed to new feeds, they usually elicit a neophobic response caused by fear of novelty, which is reinforced by the new environment. This response causes a reduction in consumption, growth, and health, and behavioral changes, which affect animal welfare (Oostindjer *et al.,* 2014).

The contrasted variable in this study was the moisture content of the diets achieved by adding CGSR+AW to the total ration. The DM value of CGSR+AW (757.6 g/kg) is 162.3 g/kg lower than that of CR (919.9 g/kg), which characterizes it as making CGSR+AW the diet with the highest moisture content. According to our hypothesis, this criterion should satisfy the preference of piglets for a humid diet, in addition to offering different palatability characteristics with silage supply. However, the intake-related behaviors observed in the present study demonstrate that piglets preferred to consume CR, at a rate of 56.39% of the total intake (Fig. 2). In the free-choice phase, preference for CGSR+AW was lower than the neutral value of 50%, which may indicate a low preference for diets containing CGSR+AW compared to diets with a dry corn grain base (Fig. 2c). According to Solà-Oriol et al. (2009), preference varies between 0 and 100%. A value equal to 50% indicates no preference for any diet, while a value greater or less than 50% indicates a significant preference or aversion to a diet, respectively.

Although the preference test enables evaluation of the animal's choice, preference strength can be assessed through the motivation test, that investigates the degree that the animal is willing to perform a task to access a resource, the resource is considered important when motivation is strong (Fraser and Matthews, 1997). In the present study, preference strength was not assessed using the motivation test; further investigations are required to assess the importance of dry grain corn or silage diets in weaned piglets.

During the free-choice phase, the consumption of CGSR+AW diet may have contributed to the decreased time allocated to feeding and facilitated ingestion, since wetter diets promote shorter eating times in pigs compared to dry diets (Zoric *et al.,* 2016). The DM content of the test diet (Table 3) was lower than that of the reference diet (757.6 vs. 919.9 g/kg), and reflects the higher moisture content in the diet that included silage. This indicates that DFI was also influenced at the free-choice phase by the ingested volume, being limited by the effect of satiety caused by greater filling of the gastrointestinal tract (De Leeuw *et al.,* 2008).

The test diet may have not been sufficient to determine feed preference, although it was composed of silage and offered modified organoleptic characteristics and improved feed digestibility (Kung Júnior *et al.,* 2018). However, we observed that the piglets did consume CGSR+AW, albeit at lower levels than the reference diet. This indicates that there was no aversion to the diet offered. Thus, an important consideration for future experiments that preference is to offer piglets the opportunity to choose among a variety of diets. During the critical weaning phase, various diets can be supplied (Middelkoop *et al.,* 2019) to facilitate feed initiation, stimulate exploratory behavior, and prompt feed intake, provided the formulation meets the nutritional requirements of piglets and does not interfere with the daily and economic management of the farms.

CONCLUSION

Including corn grain silage rehydrated with water (with or without inoculant) or acid whey in weaned piglet rations helped meet performance demands in the first few days after weaning (21–32 days of age). The effectiveness of rations, including CGSR+W and CGSR+WI, in controlling the occurrence and severity of diarrhea indicates the feasibility of replacing dry corn grain in piglet rations after the first few days post-weaning. When provided with access to a free-choice diet, piglets spent more time to feeding, and exhibited a higher DFI and preference for the reference (dry corn grain) than the test diet. However, the weaned piglets showed a greater acceptability of rehydrated corn grain silage during restricted feeding in the first weeks post-weaning. Additionally, as the first study to supply rehydrated corn grain silage to piglets during the post-weaning phase, we conclude that the diversity of diets with rehydrated corn grain silage exerted a considerable influence on feeding initiation.

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