Improving broiler performance at market age regardless of stocking density by using a pre-starter diet

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Primary Audience: Researchers, Nutritionists, Veterinarians and Poultry Producers

SUMMARY

Broiler early nutrition has caught the attention of nutritionists due to the positive correlation between early growth rate and market weight. Early nutrition strategies such as low Ca levels or the use of highly digestible ingredients (e.g., spray-dried porcine plasma [**SDPP**]) have been reported to improve gut and muscle development, immunity, and overall growth of the bird. On the other hand, recent works suggested that stocking density represents the main constrain for modern chickens to express their full genetic potential. The current study aimed to elucidate the potential effects of pre-starter feeding strategies on contrasting livestock stocking densities. The study followed a factorial design of 2 contrasting starter programs (standard [0−11d] *vs.* pre-starter [0−4 d] + standard [4−11 d]) by 3 stocking densities (low, medium, and high, 27, 33, and 39 kg/BW/m^2, respectively). Birds placed at low stocking density showed higher BW and better FCR than those at high stocking density ($P < 0.05$) being this evident only after d 28. On the other hand, birds fed on pre-starter diet led to higher BW at 4 d ($P < 0.001$) and the effects on BW were maintained until market age (+2.54%; $P < 0.05$). The pre-starter diet also led to heavier carcasses (+2.2%; $P < 0.05$), improved uniformity ($P < 0.10$) and showed a lower incidence of lameness at high stocking densities ($P < 0.05$) compared to birds fed the standard starter diet. Results of the current study confirm that, regardless of the stocking density used, pre-starter diets (0−4 d) could lead to a better early growth and market weight.

Key words: broiler, pre-starter diet, stocking density, performance

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DESCRIPTION OF PROBLEM

There is a growing interest in the use of more specific diets in young chicks to reduce stress-related factors derived from water and feed deprivation in the first hours of life. The period between hatching and farm allocation could range from 24 to 72 h. This period is considered too long for the first water and feed intake allowance (de Jong et al., 2017). Another identified stressful factor is caused by the shift of energy source in the first few hours post-hatching, going from endogenous lipids and proteins in the yolk toward a more complex

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exogenous source of carbohydrates in conventional starter diets (Leeson and Summers, 2005). And finally, the limited digestibility in young chicks due to an immature enzyme system and limited absorption capacity (Lamot et al., 2019) calls for specific pre-starter diets.

In general, pre-starter diets provide highly digestible ingredients or higher levels of nutrients (Leeson, 2008). The use of these pre-starter diets may improve early growth and health. Likewise, it could have some effects on subsequent growth phases (e.g., carryover effects). Willemsen et al. (2008) established a positive correlation between body weight at 7 d and body weight at market age. However, positive effects on bird performance are not uniquely related to improvements in nutritional aspects, other factors such as husbandry and handling of the animals play a key role growth of the bird as well. For instance, according to Leeson (2018) the main restriction for modern chickens to express their full growth potential at market age is the use of high stocking density due to its restrictive effect on feed intake.

Stocking density, calculated as the number of birds or body mass (in kg or lb) per unit of housing surface in m^2 or ft^2, represents one of the key husbandry factors that develops an important role on traits such as bird performance, animal welfare (e.g., behavior) and health status of the flock (e.g., immunity response) (Thaxton et al., 2006). Broiler producers, in general, tend to quantify their revenues by increasing the number of birds produced per barn, and thus, the more kilograms of chicken per area the higher the revenues (Cengiz et al., 2015). However, higher stocking densities, greater than 33 kg/m^2, have been associated with lower bird performance due to a higher competition for the fixed number of feeders (Leeson and Summers, 2005; Cengiz et al., 2015), greater chances for compromised immunity (Heckert et al., 2002; Thaxton et al., 2006) and, other welfare concerns, such as foot-pad dermatitis, scratches, and bruising among others (Estevez, 2007).

For logistics reasons, producers tend to minimize the number of diets offered to birds to reach market weight. This strategy, contrarily, makes it more difficult to match the exact nutritional requirements of the bird throughout the production cycle. Despite the fact the genetic companies (e.g., Ross 308 [Aviagen 2019] or Cobb 500 [Cobb-Vantress 2018]) have their own recommendations, conventional starter diets are commercially supplied during the first 10 d of life, even up to 21 d, and may not meet the optimal nutritional requirements (Gutierrez et al., 2008). Thus, following a 3-phase feeding program (starter, grower, and finisher) could be more practical than 4-phase feeding programs that use a pre-starter diet in the first days of life. Formulating starter diets represents a huge challenge for nutritionists since nutritional requirements change rapidly during the first week of life due to the continuous and rapid development of organs (e.g., those of the gastrointestinal tract or immune system) (Christensen, 2009; Lilburn and Loeffler, 2015). To overcome these issues, the use of pre-starter diets adding highly digestible ingredients may facilitate the limited digestibility of the young chicks (Barekatain and Swick, 2016).

Spray dried porcine plasma (**SDPP**) is a complex mixture of functional components including immunoglobulins, albumin, transferrin, fibrinogen, lipids, growth factors, bioactive peptides, enzymes, hormones, and amino acids commonly used in feed for young animals (Torrallardona, 2010; Pérez-Bosque et al., 2016). Porcine or bovine spray-dried plasma (**SDP**) has shown to be effective improving the intestinal barrier function, balancing the inflammatory process, reducing the incidence of diarrhea, and improving feed intake in weaned pigs (Torrallardona et al., 2003; Peace et al., 2011; Pujols et al., 2016). In young chicks, the use of SDPP has resulted in growth performance improvement (Jamroz et al., 2011; Henn et al., 2013; Campbell et al., 2019a) and has shown beneficial effects in gut health (Beski et al., 2015a). Furthermore, as observed in young pigs, the benefits of adding SDP in chick diets are improved when birds are growing under challenging conditions (Campbell et al., 2006; Beski et al., 2016; González-Esquerra, 2019b). Moreover, according to Walters et al. (2019) SDP can be considered as an alternative to reduce the use of non-therapeutic antibiotics, replacing the use of bacitracin methylene disalicylate in the diet.

During the incubation period, the embryo has limited access to minerals, except Ca, which is absorbed from the eggshell and incorporated in the yolk and embryonic tissues (Johnston and Comar, 1955). The yolk is the main source of phosphorus (**P**) necessary for the development of the skeleton and at hatching time its storage is minimal (Yair and Uni, 2011; Li et al., 2014). This physiological limitation could delay bone mineralization after hatching, especially when chickens are fed a conventional starter diet with Ca levels ranging from 9 to 10.5 g/kg, due to the well-known interference of Ca in P availability (Driver et al., 2005). In this sense, Mansilla et al. (2020) showed that reducing the Ca content in pre-starter diets (0−4 d) can increase early growth without compromising bone mineralization.

The current study aimed to investigate whether providing low Ca, SDPP and highly digestible ingredients such as micronized soy or maize flakes in the pre-starter period (0−4 d) could result in better growth at market age and, more particularly, if such strategies could alleviate somehow the problems associated with high stocking densities.

MATERIALS AND METHODS

Animals and Housing

The present study was approved by the Trouw Nutrition Animal Care Committee and followed recommendations of the Castilla-La Mancha Animal Welfare department (Boletín Oficial del Estado, 2013).

A total of 1,504 one-day-old Ross 308 male broiler chicks (SADA Inc., Alcala del Rio, Seville, Spain), vaccinated against Marek, Coccidiosis, Infectious Bronchitis and Gumboro diseases, were randomly placed in 48 identical pens with a total area of 3.06 m^2 (2.91 m × 1.05 m). In an attempt to achieve contrasting stocking densities 26, 31, or 37 birds were placed per pen to achieve an expected density of 27, 33, and 39 kg BW/m^2 respectively at 42 d. The floor area occupied by feeders was not considered on measuring the expected density. During the first 5 d, the diets were distributed on feed trays. From 3 d onward, additionally 1 tube feeder was used. All pens were equipped with 4 functional nipple drinkers, and fresh wood shaving was used as bedding material. Water and feed were offered *ad libitum* throughout the entire production cycle. The barn temperature was gradually decreased from 32°C at placement to 21°C at 42 d and the light schedule decreased from 24 h light for the first 3 d to 16:8 (light:dark) thereafter.

Experimental Design

A total of 6 experimental treatments were tested in 2 starter programs (standard [0−11 d] *vs.* pre-starter [0−4 d] + standard [4−11 d]) × 3 stocking densities factorial arrangement. The target stocking densities, were established, based on the expected BW at the end of the cycle (42 d), as follows: low stocking density at 27 kg BW/m^2, the medium stocking density at 33 kg BW/m^2 (or standard stocking density according to the Boletín Oficial del Estado, 2010) and the high stocking density at 39 kg BW/m^2, which was estimated to be achieved with 26, 31, and 37 birds/pen, respectively. The experimental period lasted for 42 d and animals underwent a 3- or 4-phase feeding program. The effect of dietary treatments, stocking density and their interaction was evaluated for broiler performance, flock uniformity, and carcass traits.

Experimental Diets

Birds received 2 contrasting starter programs (standard starter or pre-starter) from placement up to the fourth day, both feeds were formulated to be iso-energetic, but formulation did not result in similar digestible amino acid contents between the two diets. Outside of the reduced Ca in the pre-starter diets, formulation was done to meet or exceed the CVB recommendations (Centraal, 2018) for broilers. Subsequently, all birds received a common 3-phase feeding program: Starter (from 4 to 11 d), Grower (from 11 to 28 d) and Finisher (from 28 to 42 d) based on the CVB recommendations. Feed-form presentation was 2 mm pellet for pre-starter and starter phases, and 3.5 mm pellet for grower and finisher phases. Pre-starter and standard starter diets were formulated based on a maize-soybean meal-wheat diet and supplemented with

NSPs enzymes (Axtra XB TPT containing 1,220 U xylanase and 152 U β-glucanase, DuPont, Marlborough, UK) and 1,000 FTU/kg of phytase enzyme (Phyzyme XP TPT, DuPont, Marlborough, UK) to achieve a digestible P level of 4.6 g/kg. Grower and finisher diets were supplemented with equal NSPs enzymes as pre-starter and starter diets, and 500 FTU/kg of phytase enzyme. The pre-starter diet was formulated with a lower Ca level relative to the standard starter diet (4 g/kg *vs.* 10 g/kg Ca, respectively). Additionally, the pre-starter diet was formulated using a concentrate that contained highly digestible

Table 1. Ingredient composition and calculated nutrient content of pre-starter (0−4 d), starter (0−11 d), grower (11−28 d), and finisher diets (28−42 d).

Ingredient, %[1]	Pre-starter	Starter	Grower	Finisher
Maize	29.82	51.45	45.00	7.00
Wheat	12.00	12.00	21.95	62.62
Soybean meal (47% CP)	16.52	22.70	20.19	18.86
Soya oil	2.068	2.710	3.323	4.810
Soy protein concentrate (63% CP)	7.00	7.00	6.90	4.55
concentrate[2]	30	-	-	-
Salt	0.091	0.091	0.180	0.181
Mono-Ca-phosphate	0.213	0.369	0.399	0.035
Mono-Na-phosphate	0.767	0.765	-	-
Calcium carbonate	0.124	1.615	0.654	0.555
Sodium bicarbonate	-	-	0.221	0.205
L-Lysine HCl (98%)	0.189	0.215	0.201	0.208
DL-Methionine (99%)	0.336	0.245	0.233	0.222
L-Threonine (98%)	0.072	0.040	0.049	0.054
Vitamin and mineral premix[3]	0.500	0.500	0.500	0.500
Commercial phytase[4]	0.200	0.200	0.100	0.100
Commercial NSPase[5]	0.100	0.100	0.100	0.100
TOTAL	100.00	100.00	100.00	100.00
	Calculated nutrient and energy content of the diet, %[6]			
AME, kcal/kg	2,850	2,850	2,925	3,000
Moisture	10.9 (9.4)	11.4 (7.5)	10.6 (9.3)	8.9 (8.8)
Ash	4.7 (4.5)	6.1 (5.9)	4.5 (4.2)	3.8 (3.9)
CP	24.0 (24.5)	21.0 (21.7)	19.5 (19.4)	18.3 (19.7)
EE	4.7 (3.7)	5.4 (4.7)	5.9 (4.9)	6.4 (5.9)
CF	2.4 (2.2)	2.5 (2.3)	2.9 (2.7)	2.8 (2.8)
Lys, g/kg	15.03	12.78	11.87	11.09
Met, g/kg	6.76	5.60	5.30	5.02
Met + Cys, g/kg	11.03	9.06	8.60	8.30
Thr, g/kg	10.17	8.28	7.86	7.36
Ca	0.400 (0.420)	1.000 (0.975)	0.550 (0.530)	0.450 (0.435)
P	0.602 (0.593)	0.603 (0.640)	0.598 (0.415)	0.530 (0.330)
Dig P	0.460	0.460	0.350	0.290
Inositol P	0.238	0.227	0.226	0.235

[1]Units per all items unless specified.
[2]A protein-energy concentrate that contains SDPP, sugar, maize flakes, and micronized soy. Analyzed nutrient values of concentrate: AME (calculated) 2,938 kcal/kg, 9.6% Moisture, 3.2% Ash, 24.9% CP, 2.3% EE, 2.2% CF, 0.290% Ca and 0.345% P.
[3]Provided per kg of complete diet: 10,000 IU vitamin A (trans-retinyl acetate), 2,500 IU vitamin D3 (cholecalciferol), 50 IU vitamin E (all-rac-tocopherol-acetate), 2.0 mg vitamin B1 (thiamine-mononitrate), 6 mg vitamin B2 (riboflavin), 40 mg vitamin B3 (niacin), 4.0 mg vitamin B6 (pyridoxine HCl), 25 mcg vitamin B12 (cyanoco-balamin), 2.0 mg vitamin K3 (bisulfate menadione complex), 10 mg pantothenic acid (d-Ca pantothenate), 1.0 mg folic acid, 150 mcg d-biotin, 0.25 mg Se (Na_2SeO_3),1.0 mg I, 15 mg Cu ($CuSO_4 \bullet 5H_2O$), 67.7 mg Fe ($FeSO_4 \bullet 7H_2O$), 90 mg Mn ($MnSO_4 \bullet H_2O$), and 80 mg Zn (ZnO).
[4]FTU/kg, Phyzyme® XP TPT, DuPont.
[5]U/kg, Axtra XB TPT, DuPont.
[6]Analyzed values are presented within brackets below the corresponding calculated value.

ingredients such as SDPP (Appetein GS, APC Europe S.L., Granollers, Spain), sugar, maize flakes, and micronized soy. Ingredient and nutrient composition of the diets are shown in Table 1.

Performance, Flock Uniformity, and Carcass Traits Measurements

On arrival (d 1) and at the end of each feeding phase (4, 11, 28, and 42), birds and remaining feed were weighed to determine average bird BW, average daily gain (**ADG**), ADFI, and FCR. Weight of dead birds per pen was recorded daily and used to correct FCR for each feeding phase. Birds were also weighed individually on d 4, 11, and 42 and flock uniformity was calculated as proportion of broilers within 5% of the average BW.

Mortality and lameness were recorded daily. Mortality was calculated as the ratio of dead chickens to total chickens at the beginning of the study and lameness was calculated as the ratio of lame chickens to total chickens at the beginning of the study. The designation of lameness was made according to an internal procedure considering all chicks with difficulty to walk, difficulty to stand or showing partial or total immobility as lame.

At 42 d of age, after weighing all the animals individually, 5 animals per pen were selected within ±5% of pen average BW, identified and fasted for approximately 12 to 14 h before slaughtering. Birds were electrically stunned and completely exsanguinated to death. Carcass (without neck) and breast (*pectoralis major* + *pectoralis minor*) yields were determined after a chilling process of 2.5 h.

Laboratory Analysis

Analytical determinations of feeds were performed in duplicate and according to the methods of AOAC (2012): dry matter (method 934.01), crude protein with the Dumas method using a LECO analyzer (method 968.06), crude fiber (method 962.09), crude fat (method 2,003.05), and ash content (method 942.05). The determination of Ca was carried out by spectrophotometry based on a colorimetric method (AXFLOW Method G-209-98 Rev. 2) and P determination was determined by spectrophotometry based on a colorimetric test (AXFLOW Method G-103-93 Rev. 10).

Statistical Analysis

Main effects (diets and stocking densities) and the interaction were analyzed using the MIXED procedures of SAS (v. Studio, SAS Institute Inc., Cary, NC). The blocking factor (location in the barn) was included in the model as random effect. Differences among means were identified using Tukey multiple comparisons ($P < 0.05$). Mortality statistical analysis was performed as a binomial distribution using the GLIMMIX procedure of SAS studio.

RESULTS AND DISCUSSION

The current study reveals that there was no interaction ($P > 0.100$) between the diets up to 4 d and the stocking density in broiler performance along the whole cycle. In contrast, main factors separately did affect broiler performance (Table 2).

The negative impact of stocking density on animal performance at market weight because of the lower feed intake at higher stocking densities, due to the difficult access to feeders or competition among birds, was not observed.

Only during the first 4-d phase (Table 2), broilers reared at higher stocking densities reduced ($P < 0.05$) ADG and ($P < 0.001$) ADFI and showed poor ($P < 0.001$) FCR. Bird performance in the period from 4 to 28 d did not appear to be negatively influenced by increasing the stocking density, but birds did reach a lower ($P < 0.05$) final BW at 42 d and worse ($P < 0.001$) FCR compared to those at lower densities. These findings are similar to those reported in previous studies (Feddes et al., 2002; Dozier et al. 2006; Zuowei et al., 2011; Simitzis et al., 2012).

In the present study, stocking density did not affect ($P > 0.05$; Table 2) ADFI but had negative effects on FCR and ADG during the finisher phase and for the overall rearing period ($P < 0.05$). Additionally, no significant variation in any performance measurements was observed between medium stocking density and low stocking density, or between medium

Table 2. Influence of the diets (0–4 d) and stocking density on growth performance of broiler chickens from placement in the barn to 42 d of age.

	Diet (0–4 d)			Stocking density (kg BW/m^2)				*P*-value		
	Standard	Pre-starter	SEM[1]	High (39 kg BW/m^2)	Medium (33 kg BW/m^2)	Low (27 kg BW/m^2)	SEM[2]	Diet	Density	Diet × Density
0 to 4 d										
BW 0 d, g	42.7	42.6	0.10	42.7	42.7	42.6	0.14	0.596	0.784	0.887
BW 4 d, g	99.7	104.7	0.91	101.7	103.1	101.8	0.95	<0.001	0.080	0.599
ADG, g	14.2	15.5	0.22	14.7^{b}	15.1^{a}	14.8^{ab}	0.23	<0.001	0.035	0.446
ADFI, g	14.2	14.3	0.19	13.7^{c}	14.3^{b}	14.7^{a}	0.20	0.897	<0.001	0.591
FCR, g/g	1.000	0.917	0.0039	0.932^{b}	0.945^{b}	0.997^{a}	0.005	<0.001	<0.001	0.590
4 to 11 d										
BW 11 d, g	327.2	340.2	3.61	333.0	335.5	332.6	3.83	<0.001	0.610	0.224
ADG, g	32.5	33.6	0.41	33.0	33.2	33.0	0.44	0.001	0.842	0.231
ADFI, g	36.0	37.4	0.37	36.4	37.0	36.7	0.40	<0.001	0.327	0.210
FCR, g/g	1.107	1.114	0.0044	1.102^{b}	1.115^{b}	1.114^{b}	0.0049	0.166	0.046	0.725
11 to 28 d										
BW 28 d, g	1,489	1,524	20.3	1,498	1,516	1,506	21.2	0.006	0.475	0.523
ADG, g	68.3	69.6	1.01	68.5	69.4	69.0	1.05	0.042	0.490	0.687
ADFI, g	97.7	99.6	1.22	98.2	99.3	98.5	1.27	0.019	0.438	0.636
FCR, g/g	1.431	1.431	0.0046	1.433	1.432	1.428	0.0054	0.925	0.738	0.817
28 to 42 d										
BW 42 d, g	2,790	2,857	24.4	2,772^{b}	2,838^{ab}	2,860^{a}	27.5	0.011	0.021	0.257
ADG, g	92.9	95.2	1.04	91.0^{b}	94.4^{ab}	96.7^{a}	1.28	0.126	0.012	0.387
ADFI, g	160.8	163.9	1.66	160.6	163.8	162.7	1.94	0.127	0.418	0.100
FCR, g/g	1.733	1.724	0.0148	1.766^{a}	1.736^{a}	1.683^{b}	0.0164	0.511	<0.001	0.344
0 to 42 d										
ADG, g	65.5	67.1	0.63	65.1^{b}	66.7^{ab}	67.2^{a}	0.71	0.016	0.030	0.263
ADFI, g	99.9	102.0	1.01	100.0	101.7	101.1	1.10	0.023	0.259	0.290
adj FCR[3], g/g	1.430	1.400	0.0098	1.446^{a}	1.412^{ab}	1.386^{b}	0.0118	0.032	0.003	0.354

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; BW, body weight; FCR, feed conversion ratio.

[a,b,c]Values in the same row without a common superscript letter significantly differ, $P \leq 0.05$. Values are least square means and pooled SEM

[1]Standard error of the mean: (n = 24 replicates).

[2]Standard error of the mean: (n = 16 replicates).

[3]Calculate FCR adjusted to 2.5 kg on global period (FCR0-42 d = FCR0 to 42_d-(avBW42_d/1,000 − 2.5) × 0.331).

stocking density and high stocking density for the overall period ($P > 0.05$). Similar findings were also observed by McKeith et al. (2020), who reported no significant differences between standard densities (0.23 m^2 per bird) and low stocking densities (0.27 m^2 per bird) on FCR and BW. Consequently, increasing the available surface per broiler did not significantly influence the FCR or BW (0−42 d).

Despite the higher ($P < 0.05$; Table 2) BW observed at lower stocking density at 42 d of age, no differences ($P > 0.05$) in feed intake were observed throughout the whole cycle, with the exception of the first week of life, when chicks at low stocking density consumed more feed than chicks at high stocking densities ($P < 0.001$; Table 2). Thus, the initial hypothesis of having limited access to feeders due to increased stocking density as well as the competition between birds to reach the feeders was rejected and might not well be the explanation. Lana et al. (2001) observed a similar response regarding increased feed intake during the early period (1−7 d of age) in those birds at low stocking densities (10 birds/m^2) compared to those at high stocking densities (16 birds/m^2) and attributed the latter fact to environmental comfort, litter, and air quality. Conversely, Guardia et al. (2011) found an increase in feed intake during the starter phase on those birds at higher stocking density and hypothesized that untrained chicks could more easily find feed or water in denser flocks than in flocks at low stocking density. However, no effect on ADFI as birds grew older was observed, which is in agreement with our results and Mehmood et al. (2014).

According to Feddes et al. (2002) and Bessei (2006) the decreased final BW in birds at high stocking density may be explained by the close proximity of the birds during growth, and therefore not being able to dissipate the metabolic heat. Furthermore, the aggravated lameness percentage observed mainly around market age may not be the main factor contributing to the decreased final BW of birds at high stocking density.

In the present study, stocking density and diets up to 4 d did interact on lameness percentage. Birds at high stocking density and fed on pre-starter diet reduced lameness percentage ($P < 0.05$) compared to those fed on standard starter diet (Figure 1). The benefits of SDPP in performance were evident and the ability to modulate the immune response, reducing expression of pro-inflammatory cytokines under challenging conditions or physiological stress, has been demonstrated in weaned piglets (Bosi et al., 2004; Pérez-Bosque et al., 2016) and in broiler chickens (Campbell et al., 2006; Beski et al., 2016). Campbell et al. (2012) found that increasing stocking density and supplementing the diets with SDPP under coccidia vaccination challenge reduced mortality and improved broiler performance (BW and FCR). Thus, pre-starter diets containing SDPP might alleviate the percentage of lameness under stressful circumstances such as high stocking densities. Nevertheless, Zuowei et al. (2011) associated the aggravated leg weakness with

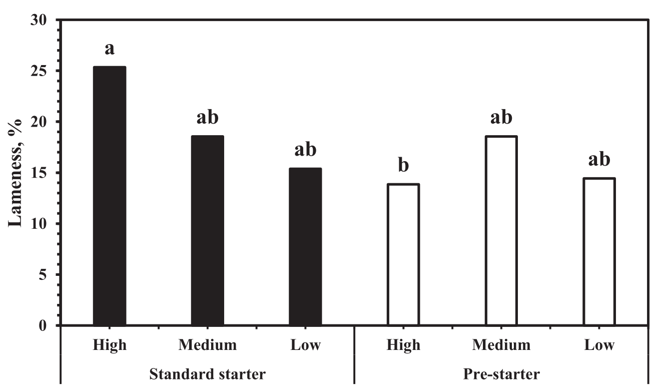

Figure 1. Influence of the diets (0 to 4 d) and stocking density on Lameness percentage in the overall period (0 to 42 d). Values are least square means.

increased feed intake in broilers at high stocking density from 36 to 42 d of age.

Previous studies demonstrated the positive effect of feeding broilers with a pre-starter diet during the first hours or days of life on their performance parameters up to market age (Saki, 2005; Hooshmand, 2006; Willemsen et al., 2008).

Many nutritional strategies have been studied with the aim of improving broiler performance during the first 7 d of age showing higher live weight, greater feed intake and better feed efficiency, but without any influence in the subsequent phases (Noy and Sklan, 2002; Longo et al., 2007). Otherwise, our work confirms the better performance of broilers fed pre-starter diets for a period of only 4 d. Body weight and FCR significantly improved ($P < 0.001$) after completing the 4-d phase and the differences observed in BW were maintained during the subsequent phases (Table 2). These results are supported by the findings of Beski et al. (2015b), and González-Esquerra et al. (2019a), who reported that supplementing broiler starter diets with SDPP improved BW in the first 10 d and throughout the study, even when all birds were fed the same common grower and finisher diets. Some studies established that a functional protein such as SDPP, which can mediate immune function and response by alleviating stressful conditions, results in improved overall performance (Campbell et al., 2019b; Walters et al., 2019). The present study confirmed the beneficial effects of SDPP supplementation to the pre-starter diets of broiler chickens.

Feed intake was not affected at the 4-d phase, but the efficiency of feed conversion improved ($P < 0.001$) with the pre-starter diet (Table 2). This was in line with the findings of Beski et al. (2015b), who observed no differences in feed intake up to 35 d between broilers fed on diets that contained 2% SDPP and control birds, but the supplementation of SDPP shows better growth at the end of the cycle. Similarly, King et al. (2005) also found that SDPP improved FCR during the first 14 d of age. However, in contrast to these results, Ullah et al. (2012) concluded that the reason for the improvement in final BW may be due to a higher feed intake during the first week of birds fed on a pre-starter diet with higher energy and optimum lysine level.

The present study reduced dietary Ca content from 10 g/kg of the standard starter diet to 4 g/kg in the pre-starter diet, in line with Torres et al. (2018). Sebastian et al. (1996) found that increasing dietary Ca from 6 to 12.5 g/kg impaired BW at 21 d, Paiva et al. (2013) showed that increasing dietary Ca from 6 to 9 g/kg reduced weight gain at 21 d, and Mansilla et al. (2020) found that increasing dietary Ca content from 4 to 10 g/kg the first 4 d reduced ADG. Mansilla et al. (2020) also found that tibia ash content decreased at 4 d by lowering Ca levels but did not affect bone health during grow-out period. Thus, higher levels of Ca needed for optimal bone mineralization could interfere P availability for other tissues, nutrient digestibility and therefore broiler performance (Driver et al., 2005; Paiva et al., 2013; Li et al., 2017). On the other

Table 3. Influence of the diets (0−4 d) and stocking density on carcass traits of broiler chickens at 42 d.

	Diet (0−4 d)			Stocking density (kg BW/m^2)				*P*-value		
	Standard	Pre-starter	SEM[1]	High (39 kg BW/m^2)	Medium (33 kg BW/m^2)	Low (27 kg BW/m^2)	SEM[2]	Diet	Density	Diet × Density
BW 42 d[3], g	2,790	2,861	28.9	2,772[b]	2,838[ab]	2,866[a]	31.9	0.014	0.024	0.325
Carcass weight, g	1,869	1,910	21.4	1,852[b]	1,894[ab]	1,922[a]	23.5	0.042	0.020	0.208
Carcass yield, %	67.03	66.70	0.16	66.88	66.67	67.05	0.196	0.178	0.434	0.098
Breast weight, g	520.5	527.7	7.49	509.4[b]	525.0[ab]	537.9[a]	8.39	0.344	0.015	0.362
Breast yield, %	18.67	18.42	0.127	18.40	18.47	18.77	0.164	0.248	0.312	0.765

[a,b,c]Values in the same row without a common superscript letter significantly differ, $P \leq 0.05$. Values are least square means and pooled SEM.

[1]Standard error of the mean: (n = 24 replicates).

[2]Standard error of the mean: (n = 16 replicates).

[3]Five animals per pen were selected within ±5% of pen average BW.

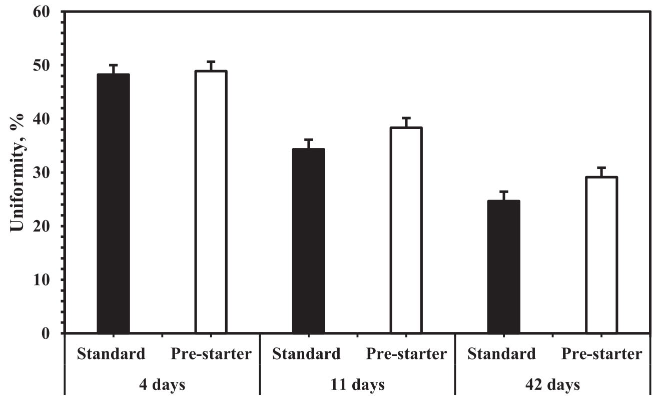

Figure 2. Influence of the diets (0 to 4 d) and age on Flock Uniformity percentage. Values are least square means ± SE.

hand, according to Mansilla et al. (2020) birds fed low Ca diets showed higher feed intake during the pre-starter phase which was responsible for the improvement of BW during the first week.

In our study birds fed the pre-starter diet had higher carcass weight (+2.2%, $P < 0.05$), which might be related to the final BW (+2.54%, $P < 0.05$) results (Table 3). Abdulla et al. (2017), reported that birds fed 15 g/kg Ca had lower carcass weight compared to those fed 10 or 12.5 g/kg Ca and also concluded that there was a possible relation with BW, lower in those birds fed 15 g/kg Ca. Furthermore, Arce-Menocal et al. (2021) found improved carcass weight and yield feeding 2% SDPP up to 7 d of age. Likewise, birds at lower stocking density had higher ($P < 0.05$) carcass and breast weight (Table 3). Similarly, Dozier et al. (2006) reported lower carcass and breast fillet weight due to an increase of stocking density. It was expected that crowded birds would not grow to their full potential, which might be also related to final BW. The breast and carcass yields were not significantly ($P > 0.05$) affected by the diets up to 4 d, neither by stocking density. This was in agreement with previous works including SDPP (Longo et al., 2007; Henn et al., 2013;

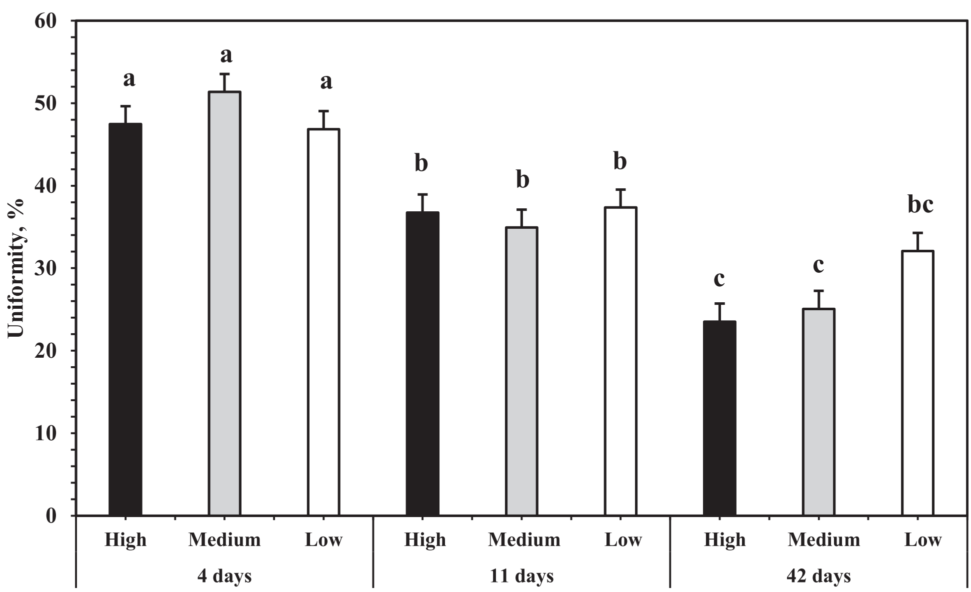

Figure 3. Influence of stocking density and age on Flock Uniformity percentage. Values are least square means ± SE.

Beski et al., 2015b) or different stocking densities (Feddes et al., 2002; Zuowei et al., 2011; Simitzis et al., 2012). In contrast, Cengiz et al. (2015) observed a decrease in relative carcass yield in birds at higher stocking density which was linked with a reduction in feed intake and thus, a lower weight gain.

In the current study flock uniformity was not influenced ($P > 0.05$) by stocking densities (Figures 2 and 3). This result is not consistent with Feddes et al. (2002) who found higher variability in animals at low stocking density which could have resulted in greater floor space allowing fast-growing birds to grow to their full potential. In contrast, Mehmood et al. (2014) reported that birds reared at lower stocking densities exhibited better uniformity concluding that having ample space could satisfy the natural behavior of the birds resulting into better uniformity. However, in the current study, uniformity decreased across ages ($P < 0.001$; Figures 2 and 3), and stocking density and age did interact ($P < 0.05$) on uniformity (Figure 3). Hence, our results are more in line with Mehmood et al. (2014), as the decrease in uniformity at 42 d was not as severe for birds at low stocking density. On the other hand, there was a tendency ($P = 0.071$; Figure 2) to improve uniformity in birds fed the pre-starter diet (38.78%) compared to those fed on the standard starter diet (35.74%). Similar findings were observed by Sklan et al. (2000) who reported improved uniformity in birds up to 21 d with a diet for the first week whereas, Bregendahl et al. (2005) and Henn et al. (2013) asserted that the inclusion of SDPP did not increase uniformity.

CONCLUSIONS AND APPLICATIONS

Despite the negative impact on ADG and FCR of high stocking densities, feeding birds with a pre-starter diet low in Ca (4g/kg), containing highly digestible ingredients and SDPP as a functional protein from 0 to 4 d of age led to an improvement in broiler performance with carry over effects up to market age regardless of the stocking density.

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