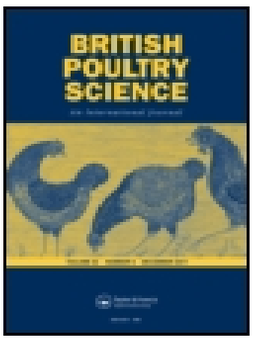

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Efficacy and stability of a novel silica supplement for improving bone development in broilers

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ABSTRACT

1. The essentiality of silicon for skeletal development has been established, but the adequacy of bioavailable silicon supply in broiler diets has not been considered for 30 years, despite average daily weight gain of birds increasing by almost a third over that time. Therefore, two studies were undertaken to investigate whether modern strains of broiler chicken benefit from diet supplementation with bioavailable silica.
2. Trial 1 was a 2x2x2 factorial study where six replicate pens of seven chicks were fed one of the eight freshly prepared diets from hatch to 21 days of age, with bodyweight gain and feed intake recorded weekly. Diets combined the following factors: silicon supplement fed at 0 ppm or 1000 ppm, phytase levels of either 0 FTU/kg or 1500 FTU/kg and either 0.6% or 0.7% Ca. Tibia were analysed for bone breaking strength, extent of tibial dyschondroplasia and feet measured for bone ash and pododermatitis score.
3. Trial 2 used a 0.7% Ca with 1500 FTU phytase diet as the control and compared this with the same diet containing either 1000 ppm silicon (MONO-Si) freshly added each week or 1000 ppm silicon added in a single, advance-prepared batch per feeding phase. Each diet was fed to nine pens of seven birds from 0 to 35 d with feed consumption and weight recorded weekly. Two birds per pen were euthanised on d 14, 21 and 35 and tibias collected for measurement of bone breaking strength, ash and mineral content. Serum was collected for Si content.
4. Univariate analysis of means from each trial showed that silica supplementation improved bird weight gain over the starter phase, though there was no effect on feed conversion.
5. Bone strength improved with added silica in both studies, without affecting bone mineral content; indicating that modern strains of broiler may require dietary supplementation with bioavailable silicon.

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Silica; broilers; bone; mineralisation; phytase

Introduction

Increases in poultry growth rate have contributed to higher incidence of skeletal issues, leading to lameness, mortality and processing difficulties. Skeletal issues may be substantially mitigated by optimisation of nutrient supply, and, in particular, the use of exogenous phytase enzymes to render phytate-bound dietary phosphorus more available, which facilitates improved bone development in poultry. More than 60% of all poultry feeds produced globally contain this enzyme (Lei et al. 2013). However, lameness and skeletal issues persist as a challenge to the poultry sector. Poultry studies in the 1970s demonstrated the clear effect of silicon deficiency on skeletal formation in poultry (Carlisle 1972), but at that time, it was necessary to artificially create silicon-free diets to demonstrate the biological effect of insufficient silicon. Modern strains of broiler chicken not only have greatly increased daily nutrient requirements compared to the strain studied by Carlisle (Applegate and Angel, 2014) but they are also reported to exhibit a very different bone structure (Williams et al. 2000). From these changes, it may be that modern strains of broiler chickens are now commonly silicon deficient.

The capacity of silicon to influence mammalian bone growth and remodelling in non-deficient situations is now well established in research settings (Jurkic et al. 2013). However, the physico-chemical properties of silicon result in highly stable silicates that are highly resistant to solubilisation (Van Dyck et al. 1999), so most commercial forms are not bioavailable at neutral pH. Nonetheless, some silicon supplements have been shown to reduce lameness through an entirely different mechanism, *i.e.* the adsorbent properties of zeolites appear to alter the functional quality of excreta and, subsequently, bedding quality, resulting in reduced lameness associated with pododermatitis (Tran et al. 2015).

To date, the capacity of silicon to remodel bone in avian species has not been exploited, due to the difficulty in presenting it in a bioavailable form. For silicon to be bioavailable, it must be presented at the target tissue in its monomeric form, *i.e.* orthosilicic acid (Jurkic et al. 2013). Contact with stomach acid can release small amounts of orthosilicic acid, which may partially explain some of the contrasting results reported in association with feeding zeolites to poultry (Ballard and Edwards 1988; Leach et al. 1990; Eliott and Edwards 1991). These supplements are slightly solubilised at low pH, but pH increases with passage through the digestive tract which results in condensation of silicon to form polymers, which then precipitate into a form where the size and charge is too large for absorption into the distal gastrointestinal tract (Jugdaohsingh 2007).

A novel form of silicon has been developed (Belton and Perry 2016) which maintains the monomeric form of the

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mineral in a non-caustic format that may be incorporated into feed. This silica (MONO-Si) is based on stabilised orthosilicic acid, in a form where it can maintain its small molecular size and can therefore readily absorbed in the small intestine (Rabon et al. 1995). One small-scale, early study showed promising effects on poultry lameness (Short et al. 2011) and evidence for intestinal absorption of MONO-Si has been shown *via* a dose dependent increase in serum silica content (Short et al. 2011). However, this form of silicon remains largely un-investigated, other than in a recent, fundamental study (using phytase-free diets) that showed an increase in bone ash and silica content and an unexpected improvement in bird performance (Scholey et al. 2018a).

In vitro studies have demonstrated the capacity of silicon to influence collagen assembly (Eglin et al. 2006). Human osteoblast-like cells have shown that silicon, in culture medium, increases collagen type 1 synthesis (Reffitt et al. 2003). More recently, studies using human mesenchymal stem cells demonstrated a dose-dependent induction of key marker genes of osteogenesis in response to silica (Martín-Moldes et al. 2018). Through this capacity to increase the rate of collagen matrix formation in bone, it is possible that bioavailable silicon may interact with phytase in affecting bone mineralisation. However, the possible impact of Ca as a third, interactive factor must be considered, as research has suggested that reducing dietary Ca levels below 1% increases efficacy of phytase (Akter et al. 2016). Specifically, higher Ca diets have been shown to alter the breakdown of inositol phosphate (IP) esters; potentially creating more intermediate esters, IP3 and IP4, which have their own detrimental effects on digestibility of nutrients required for optimal bone formation (Bedford and Rousseau 2017).

The main aim of this study was to investigate whether previously reported effects of a novel silica supplement (MONO-Si) on performance and bone parameters in young broilers are affected by dietary phytase inclusion or when levels of Ca are reduced. Further, the stability of any new supplement is vital to allow for transport and storage when produced commercially. Stability studies on MONO-Si at NTU have shown that the material is stable in its pure form, but stability when included in a diet mix was less certain, due to the potential for condensation of the monomeric Si to a larger, less bioavailable form. Previous studies with MONO-Si were based on weekly diet manufacture, so it is important to test whether large-scale manufacture is viable. Therefore, the secondary aim of this investigation was to determine the efficacy of MONO-Si when made in batches compared with weekly manufacture on birds grown to slaughter age.

Materials and methods

Study 1: effect of phytase and calcium level on efficacy of MONO-Si supplementation

Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by Nottingham Trent University's College of Science ethical review committee (approval code ARE15).

Male, Cobb 500 broilers (n = 336) were obtained from a commercial hatchery on the day of hatch. Chicks were individually weighed and placed into 0.64 m^2 floor pens in groups of seven birds per pen, bedded on clean wood shavings. Pens were randomly allocated to one of the eight dietary treatments, giving six replicate pens per treatment. On arrival, birds were allowed access to feed and water *ad libitum*. The room was maintained at an initial temperature of 31°C on d 1 and reduced to reach 21°C by d 21. Lighting was provided for 24 h on d 1, with darkness increasing by 1 h/d until 6 h of darkness was reached, which continued throughout the rest of the study.

Diets were formulated with wheat and soya bean meal and manufactured in-house as mash. Nitrogen content of the diets was determined using a combustion analyser (Dumatherm N Pro, Gerhardt Analytical Systems, Germany) then multiplied by 6.25 to derive crude protein content. Ether extractable fat content and crude mineral content were analysed according to AOAC (2000) (method 945.16 and 942.05, respectively). Following an *aqua regia* digestion step (AOAC 985.01) Ca, P and Si content were analysed using an inductively coupled plasma optical emission spectrometer (ICP-OES, Avio200 Model – 725 radial view, Perkin Elmer, Beaconsfield, UK).

The trial was designed as a 2x2x2 factorial study with the silica supplement (MONO-Si) fed at 0 ppm or 1000 ppm, phytase level at either 0 FTU/kg or 1500 FTU/kg (Quantum Blue, ABVista, UK) and either 0.6% or 0.7% dietary Ca, which was achieved by increasing limestone inclusion level in the 0.7% Ca diets. Diets including the silica supplement were manufactured weekly. The silica supplement (MONO-Si) was created as described by Belton and Perry (2016). Briefly,; sodium metasilicate (Fisher Chemicals, UK) was mixed with an equal quantity of anhydrous citric acid (Fisher Chemicals, UK) and suspended in soya oil prior to dietary addition and mixing for 5 min using a ribbon mixer (Rigal Bennett, UK). Ingredient and nutrient content of experimental diets are shown in Table 1.

Bird weight and feed consumption were recorded weekly by pen, with six pens (with pen designated as the experimental unit) per treatment. On day 21, two birds per pen were euthanised and, from each bird, the tissues described below were excised combined to form one experimental unit. Feet were removed at the tibial-tarsal joint and dried until a constant weight was achieved at 105°C before ashing for 13 hours at 650°C (Garcia and Dale 2006). Tibias were removed at the femoral-tibial joint (one per bird) and flesh removed by hand (Shaw et al. 2010) before the bones were assessed for tensile strength using a TA.XT plus texture analyser (Stable Microsystems, Guildford, UK) set up with a 50 kg load cell and three-point-bend fixture (Scholey et al. 2018b). The texture analyser was set to measure force under compression, test speed was set at 1 mm/sec, and trigger force was set at 7 g (0.069 N). The broken tibias were then autoclaved to remove any remaining flesh, but cartilage caps were left on and dried for 48 hours at 105°C. Feet (tarsometatarsus together with all toes) were removed and left with skin and soft tissues attached for ash determination. Dried tibias were ashed at 650°C for 13 hours. Both foot and tibia ash were calculated as a proportion of ash weight to dry weight.

All data were assessed for normality by Kolmogorov–Smirnov tests and Levene's test for equal variances was applied to test the assumptions required for analysis of variance (ANOVA). Multiple ANOVA was used to determine the effect of Ca:P ratio, phytase and silica inclusion on response variables. The statistical model included Ca:P, phytase and silica to investigate all two- and three-way interactions with statistical significance declared at $P < 0.05$.

Table 1. Ingredient and nutrient content of experimental diets used in Trial 1.

	Non-silica supplemented diets				Silica-supplemented diets			
	0.6% Ca diets		0.7% Ca diets		0.6% Ca diets		0.7% Ca diets	
Ingredient (g/kg unless Specified)	No Phy[3]	Phy	No Phy	Phy	No Phy	Phy	No Phy	Phy
Wheat	606.4	606.4	601.0	601.0	604.4	604.4	599.0	599.0
Rapeseed Meal	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Soybean meal[1]	287.0	287.0	288.2	288.2	287.0	287.0	288.2	288.2
Soya oil	39.8	39.8	41.5	41.5	39.8	39.8	41.5	41.5
Salt	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Sod Bicarbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Methionine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Lysine	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Threonine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Limestone	4.3	4.3	6.8	6.8	4.3	4.3	6.8	6.8
Dical Phos	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
Vit/Min premix[2]	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
Silica	0	0	0	0	2.0	2.0	2.0	2.0
Phytase (FTU)	0	1500	0	1500	0	1500	0	1500
Calculated nutrients								
Crude protein	220	220	220	220	220	220	220	220
ME MJ/kg	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6
Calcium	6.1	6.1	7.0	7.0	6.1	6.1	7.0	7.0
Total Phos	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
nPP	3.2	4.7	3.2	4.7	3.2	4.7	3.2	4.7
Av Silicon	0	0	0	0	1.0	1.0	1.0	1.0
Crude fat	55	55	56	56	55	55	56	56
Analysed nutrients								
Crude Protein	219	215	223	223	223	223	215	216
Crude fat	57	55	57	56	54	55	53	53
Crude Ash	48	47	48	49	54	56	54	50

[1]Crude protein content 48%.
[2]Vitamin and Mineral Premix content (per kg diet): Mn 100 mg, Zn 88 mg, Fe 20 mg, Cu 10 mg, I 1 mg, Mb 0.48 mg, Se 0.2 mg, Retinol 13.5 mg, Cholecalciferol 3 mg, Tocopherol 25 mg, Menadione 5.0 mg, Thiamine 3 mg, Riboflavin 10.0 mg, Pantothenic acid 15 mg, Pyroxidine 3.0 mg, Niacin 60 mg, Cobalamin 30 µg, Folic acid 1.5 mg, Biotin 125 µg.
[3]Denotes whether diets contained Quantum Blue™ Phytase (AB Vista Feed Ingredients).

Study 2: efficacy of a batch manufactured MONO-Si diet compared with weekly manufacture

Institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by Nottingham Trent University's College of Science ethical review committee (approval code ARE96).

To investigate stability of the novel silicon supplement, data from a second study (previously partially published as a comparison of the MONO-Si supplement to an existing form of silicon supplement; Scholey et al. 2018a) were analysed to compare weekly manufacture against bulk manufacture for the whole growth period. The trial was carried out with diets made using a single basal formulation in mash form for both the starter and finisher phase, containing 1500 FTU phytase and 0.7% Ca.

In total, 189 male Ross 308 birds were fed one of the three diet treatments from d 0–35 in two phases (starter day 0–21, finisher day 22–35) with feed consumption and bird weight recorded weekly. The three dietary treatments comprised a control (basal diet only), MONO-Si added weekly at 1000 ppm to basal diet ('Si weekly') or MONO-Si added at the start of each growth phase (starter and finisher) at 1000 ppm ('Si batch'). Each dietary treatment was fed to nine pens replicates (pen being the experimental unit) with seven birds placed per pen at d 0. Diet formulations and nutrient composition for the basal diet for each phase are shown in Table 2. Lighting, pen set up and temperature were all maintained as described in Trial 1.

Table 2. Ingredient and nutrient content of starter (d 0–21) and finisher (d 22–35) basal diets used in Trial 2.

Ingredient (g/kg unless specified)	Starter Basal	Finisher Basal
Wheat	631.8	717.1
Rapeseed Meal	40.0	40.0
Soybean meal[1]	259.7	183.0
Soya oil	35.6	34.5
Salt	3.0	3.0
Sod Bicarbonate	1.0	1.0
Methionine	2.8	1.5
Lysine	2.6	2.1
Threonine	0.7	0.4
Limestone	9.1	8.7
Dical Phos	8.7	3.7
Vit/Min premix[2]	5.0	5.0
Phytase[3] (FTU)	1500	1500
Calculated values		
Crude protein	210	181
ME MJ/kg	12.6	12.8
Calcium	8.0	6.5
Total Phos	6.1	4.9
nPP	4.8	3.9
Crude fat	50	49
Analysed nutrients		
Crude Protein	200	194
Crude fat	73	65
Crude Ash	42	39

[1]Crude protein content 48%.
[2]Premix content (volume/kg diet): Mn 100 mg, Zn 88 mg, Fe 20 mg, Cu 10 mg, I 1 mg, Mb 0.4 8 mg, Se 0.2 mg, Retinol 13.5 mg, Cholecalciferol 3 mg, Tocopherol 25 mg, Menadione 5.0 mg, Thiamine 3 mg, Riboflavin 10.0 mg, Pantothenic acid 15 mg, Pyroxidine 3.0 mg, Niacin 60 mg, Cobalamin 30 µg, Folic acid 1.5 mg, Biotin 125 µg.
[3]Quantum Blue™ Phytase (AB Vista Feed Ingredients).

Two birds per pen were euthanised on d 14, 21 and 35 and sampled for bone breaking strength and tibia ash, as described in Trial 1. In addition, the bone ash for each tibia was digested using *aqua regia* (a mixture of nitric acid and hydrochloric acid, optimally in a molar ratio of 1:3) and analysed for Ca, P and Si content using an Avio200 ICP-OES (Perkin Elmer, Beaconsfield, UK).

All data were analysed using SPSS (v.24). After Kolmogorov–Smirnov testing to confirm normality, statistical analysis was carried out using either one-way ANOVA or Kruskal-Wallis independent sample tests to compare performance parameters, bone measures and tibia mineral content from birds fed the different dietary treatments. Dunn's *post hoc* test with a Bonferroni correction to control the experiment-wise error was used where appropriate to elucidate differences between sources. Statistical significance was declared at $P < 0.05$.

Results

Study 1 investigated the efficacy of a novel Si supplement, MONO-Si, on performance and bone parameters in the starter period (d 0–21) in a 2x2x2 factorial design, with two Ca levels; with and without phytase added at 1500 FTU/kg diet ('superdosing'); and with and without bioavailable silicon added at 1000 ppm. Ca levels were set as one substantially, and the other slightly lower than the nutrient recommendations for the bird strain (Cobb Vantress Inc 2018), at 0.6% and 0.7% Ca, respectively).

Table 3 shows that there was a significant increase in both body weight gain (BWG; $P = 0.045$) and feed intake (FI; $P = 0.037$) in response to Si supplementation, but no effect on feed conversion ratio (FCR). Neither phytase supplementation nor a higher Ca level invoked a significant performance response in this trial. While dietary Ca level did not affect any

Table 3. Main effects of dietary phytase, silica and calcium inclusion in study 1 on performance (n = 6) from 0 to 21 days, bone ash and tibia strength in birds at d 21 days of age (n = 12 per treatment).

Treatment Factor	BWG (g)	FI (g)	FCR	Tibia strength (N)	Tibia ash (%)	Foot ash (%)
No Si	792[b]	1117[b]	1.41	142[b]	46.7	15.3
Si	826[a]	1158[a]	1.41	162[a]	47.2	15.5
P value	0.045	0.037	0.653	0.040	0.191	0.242
No phytase	798	1123	1.41	147	46.1[b]	15.1[b]
Phytase	820	1152	1.41	157	47.9[a]	15.7[a]
P value	0.228	0.347	0.336	0.312	P < 0.001	0.002
0.6% Ca	799	1129	1.42	151	46.9	15.5
0.7% Ca	819	1145	1.40	153	47.0	15.3
P value	0.709	0.805	0.349	0.870	0.654	0.417
SEM	11.6	13.3	0.011	0.67	0.34	0.14

[a–b]Means within the same column with no common superscript differ significantly (P < 0.05).
BWG: body weight gain per bird, FI: feed intake per bird and FCR: feed conversion ratio.

bone parameters, Si and phytase addition affected bone parameters in different ways. Si supplementation significantly (P = 0.04) increased bone strength at d 21, but there was no concurrent increase in bone ash. In contrast, phytase supplementation significantly increased both tibia (P < 0.001) and foot ash (P = 0.002) but did not significantly affect bone breaking strength. Increasing dietary Ca from 0.6% to 0.7% did not affect strength or bone ash.

Within the 24 probability values generated by examining the four possible interactions within the six response parameters, a significant interaction (P = 0.019) was observed between the effects of Si and Ca on FCR. Individual diet responses for each parameter are shown in Table 4.

Study 2 compared the effects of a novel Si supplement (MONO-Si), either mixed into diets on a weekly basis or made in advance as one complete batch, on broiler performance and bone parameters to 35 d post hatch. Table 5 shows that, at d 21, there was a significant improvement in BWG (P = 0.034) in birds fed batch-made silicon diets compared to birds fed the control diet, with an intermediate response seen in birds fed the weekly made, silicon-containing diets, but no difference in FI between the three diets. In contrast, cumulative performance to d 35 showed no differences between dietary treatments for BWG or cumulative FCR, but there was a significant increase in FI (P = 0.036) for birds fed the weekly made Si diet compared to the control group, with an intermediate effect observed in birds fed the batch-made Si diets.

Table 6 shows the tibia strength and ash of birds in Trial 2 at d 14, 21 and 35. There was no effect of batch- or weekly made Si supplementation on bone ash at any age. While there was no effect of either batch- or weekly made Si supplementation at d 14, by d 21 bone breaking strength was significantly (P = 0.004) higher in birds fed the batch-made silicon compared to control-fed birds, but no significant difference compared to the control group was seen in birds fed weekly made Si at this age. At d 35, the same significant (P = 0.006) increase in bone strength was seen in birds fed batch-made Si diets, and at this age, an intermediate response was seen in birds fed the weekly made Si diet.

In alignment with the ash findings, Table 7 shows there were no differences in tibia Ca or P content at day 35, but there was a significant increase in tibia silicon content of birds fed batch-made Si diets, with an intermediate response observed in birds fed weekly made Si diets.

Discussion

It has been accepted that exogenous phytases show improved efficacy in broilers fed relatively low dietary Ca levels and relatively low (less than 1.8:1) Ca:P ratios (Selle et al. 2009). However, two recent studies (Gautier et al. 2017; Lee et al. 2017) suggested that the anticipated beneficial effect of phytase on bone mineralisation does not always occur in diets with a low Ca:P ratio. The difficulty in explaining these findings may be linked to the current sector focus on provision of sufficient Ca and P for hydroxyapatite deposition in bone matrix. Little consideration has been currently given to factors affecting synthesis of type 1 collagen: the main component of the bone matrix itself. Silicon, in the form of orthosilicic acid, has been shown to stimulate type 1 collagen synthesis in osteoblast-like cells (Reffitt et al. 2003), suggesting improved supply of orthosilicic acid provides a route to improving the development of the bone matrix in fast-growing broiler chicks. This investigation highlighted the possible role of bioavailable silicon alongside phytase in low calcium diets as a key to improved bone development in fast-growing broilers.

Table 4. Dietary performance (n = 6 per treatment) from 0 to 21 d of age, bone ash and tibia strength (n = 12 per treatment) at day 21 of age in birds fed diets with and without phytase and silica at differing dietary calcium levels in Trial 1.

			Parameters					
Diets Calcium ('Ca')	Phytase ('Phy')[1]	Silicon ('Si') [2]	BWG (g)	FI (g)	FCR	Tibia strength (N)	Tibia ash (%)	Foot ash (%)
0.6%	-	-	755	1098	1.46	141	46.8	15.2
		+	786	1120	1.43	143	47.2	15.9
	+	-	814	1115	1.37	134	45.1[a]	14.8[a]
		+	812	1135	1.40	149	47.6	15.1
0.7%	-	-	808	1127	1.40	158	45.7	15.2
		+	849	1173	1.40	162	47.9	15.6
	+	-	816	1152	1.41	153	46.7	15.1
		+	832	1180	1.41	174	48.7	16.1
		P value	0.284	0.129	0.822	0.168	0.008	0.018
		SEM	21.7	25.3	0.02	13.2	0.65	0.28
Interactions	P values	Si*Phy	0.670	0.683	0.895	0.819	0.490	0.544
		Si*Ca	0.154	0.996	0.019	0.828	0.108	0.052
		Ca*Phy	0.396	0.798	0.399	0.459	0.311	0.743
		Si*Phy*Ca	0.899	0.828	0.316	0.910	0.237	0.184

[1]Quantum Blue phytase added at 1500 FTU per kg diet.
[2]MONO-Si added at 1000 ppm.
[a–b]Means within the same column with no common superscript differ significantly (P < 0.05).
BWG: body weight gain, FI: feed intake and FCR: feed conversion ratio.

Table 5. Mean per pen bodyweight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of birds in Trial 2 fed diets with and without silica, added weekly or in one batch, from days 0–35 (n = 9 per treatment).

Diet	BWG d0-21 (g)	FI d0-21 (g)	FCR d0-21	BWG d22-35 (g)	FI d22-35 (g)	FCR d22-35	BWG d0-35 (g)	FI d0-35 (g)	FCR d0-35
Control	799[b]	1096	1.38	1176	1176	1.75	1975	3140[b]	1.60
Weekly Si[1]	831[ab]	1121	1.35	1210[a]	1210	1.92	2041	3438[a]	1.68
Batch Si[2]	887[a]	1155	1.31	1168	1168	1.79	2055	3236[ab]	1.58
P value	P < 0.05	0.203	0.422	P < 0.05	0.111	0.422	0.567	P < 0.05	0.203
SEM	21.7	26.5	0.030	42.8	59.7	0.041	53.9	77.8	0.030

[1]Weekly Si diets were made fresh weekly.
[2]Batch Si diets were made in one batch at the start of the study.
[a–b]Means within the same column with no common superscript differ significantly (P < 0.05).

Table 6. Tibia ash and strength of birds fed diets with or without silica at 1000 ppm in Trial 2 (n = 18 per time point per treatment).

Diet	d14 bone ash, %	d21 bone ash, %	d35 bone ash, %	d14 bone strength, N	d21 bone strength, N	d35 bone strength, N
Control	44.0	49.2	48.9	71	191[b]	344[b]
Weekly Si[1]	45.7	50.2	48.6	72	196[b]	389[ab]
Batch Si[2]	45.8	50.5	49.3	68	243[a]	420[a]
P value	0.177	0.197	0.710	0.683	P < 0.01	P < 0.01
SEM	0.78	0.52	0.54	3.5	10.5	14.3

[1]Weekly Si diets were made fresh weekly.
[2]Batch Si diets were made in one batch at the start of the study.
[a–b]Means within the same column with no common superscript differ significantly (P < 0.05).

Table 7. Tibia mineral content (g mineral/kg dry tibia) at 35 d of age from birds in Trial 2 fed diets with and without silica at 1000 ppm, made weekly or by batch (n = 18 birds per treatment).

Diet	Tibia Si, g/kg	Tibia P, g/kg	Tibia Ca, g/kg
Control	0.15[b]	76.7	184.9
Weekly Si[1]	0.18[ab]	76.5	183.0
Batch Si[2]	0.20[a]	78.9	188.3
P value	0.032	0.102	0.212
SEM	0.012	0.86	2.07

[1]Weekly Si diets were made fresh weekly.
[2]Batch Si diets were made in one batch at the start of the study.
[a–b]Means within the same column with no common superscript differ significantly (P < 0.05).

Trial 1 investigated the efficacy of a novel dietary Si supplement (MONO-Si) on performance and bone parameters in the period from hatch to d 21 when fed in conjunction with a superdose (1500 FTU) of phytase in diets containing relatively low levels of Ca (0.6% and 0.7% respectively). This trial resulted in contrasting effects of the Si and phytase on bone parameters, whereby Si supplementation did not result in any significant effect on mineralisation at d 21, but had a significant impact in bone strength. However, the opposite response was seen with phytase supplementation, whereby bone mineralisation was significantly increased, but no significant increase in bone strength occurred. This suggested that the two supplements affected bone development through different mechanisms.

The significant increase in growth up to d 21 observed in trial 1 in birds fed MONO-Si was surprising as, although studies have commonly observed reduced growth rates of birds fed artificially low Si diets (Brossart et al. 1990; Seaborn and Nielsen 1994, no performance effect was observed when diets containing more than background levels of Si were fed (Carlisle 1980; Eliott and Edwards 1991). This profound improvement in performance (albeit in a relatively small sample size) in response to bioavailable Si supplementation suggested that modern strains of broiler chickens may be Si deficient when fed a standard broiler diet. It is accepted that rapid growth of modern broiler strains has increased requirements for a number of nutrients, compared with the strains used 30 years ago, lending support to the theory that the requirement for bioavailable Si may have increased beyond the amount naturally supplied in commercial diets (Applegate and Angel, 2014; Williams et al. 2000). This apparent under-supply of bioavailable Si is in sharp contrast to the high total Si content (4.7–6.4 g/kg) in poultry diets (Scholey et al. 2018a). Some Si is naturally hydrolysed to orthosilicic acid in the acidic environment of the digestive system and absorbed, but this is a very small amount (Reffitt et al. 2003), as, above 2 mM, silica polymerises and becomes unavailable (Iler 1978; Jugdaohsingh 2007).

Having observed a positive effect of bioavailable Si on early bone development and performance of broiler chicks in Trial 1, a second study was undertaken to determine MONO-Si effects to bird maturity at d 35 and to further test the stability of the supplement in the diet. Trial 2 showed a significant improvement in body weight gain of the Si-supplemented birds in the starter phase compared with birds fed the control diet, but the significance of this effect was lost at d 35, possibly indicating that the Si requirement for optimum growth was not met by 1000 ppm of MONO-Si, despite the positive effects of this supplementation level on tibia strength. The FI was significantly increased as the birds progressed towards slaughter weight. It was difficult to confidently predict response of birds to altered silicon supplementation level from Trial 2, as the diet specification did not match the breeder guidelines for the strain of bird used, and the number of birds in each pen (representing one replicate) was low, at three birds per pen on d 35.

Bone ash was not increased by Si inclusion at any age in either trial, warranting deeper investigation into whether supplementation with bioavailable Si affects mineral deposition as well as early development of the bone matrix. Interestingly, in Trial 2, differences between dietary treatments in bone strength were not observed until d 21, which may have been related to developmental differences in the strain of birds used in the two studies or the use of dietary Ca levels below the level recommended by the breeders for Cobb type birds in Trial 1. A key finding from Trial 2 was that it was not necessary to add MONO-Si to diets on a weekly basis. In fact, response to the batch-made Si diets was superior to the weekly made MONO-Si diets.

To conclude, these studies provided clear evidence that bioavailable Si can improve bone strength in modern broiler strains while increasing bird bodyweight in young chicks up to 21 d. Further investigations are needed to identify the mode of action of the Si in improving bird bone strength, and whether there is a definitive effect on mineral deposition in the bone.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data Availability Statement

The data that support the findings of this study are available from EJB. Restrictions apply to the availability of these data, which were used under licence for this study. Data are available from the authors with the permission of AB Vista Feed Ingredients.

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