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ABSTRACT Dietary ingredient and nutrient composition may affect the efficacy of additives in broilers. Specific feed ingredients can represent dietary challenging conditions for broilers, resulting in impaired performances and health, which might be alleviated by dietary probiotics and postbiotics. We assessed the effects of a *Lactobacilli* probiotic (**Pro**) and postbiotic (**Post**) when added to a standard (**SD**) and challenge (**CD**) diet. A completely randomized block study with 2 diets (SD, CD) and 3 additive conditions (Control, Pro and Post) involving 1,368 one-day-old Ross male broilers, equally distributed among 36 pens, from d1 to d42 was conducted. Both diets were formulated to contain identical levels of nutrients, with CD formulated to be richer than SD in nonstarch polysaccharides using rye and barley as ingredients. Readout parameters included growth performance parameters, footpad lesions score, blood minerals and biochemical parameters, and tibia health, strength, and composition. Compared to SD, CD decreased BW (1,936 vs. 2,033 g; $p = 0.001$), increased FCR ($p < 0.01$) and impaired tibia health and strength ($p < 0.05$) at d35, thereby confirming the challenging effect of CD. Pro and Post increased BW in CD (+4.7 and +3.2%, respectively, at d35; $P < 0.05$) but not in the SD group, without affecting FCR. Independently of the diet, Pro increased plasma calcium, phosphorus and uric acid at d21 (+6.2, +7.4, and +15.5%, respectively) and d35 (+6.6, +6.2 and +21.0%, respectively) ($P < 0.05$) while Post increased plasma magnesium only at d21 (+11.3%; $P = 0.037$). Blood bile acids were affected by additives in an age- and diet-dependent manner, with some opposite effects between dietary conditions. Diet composition modulated Pro and Post effects on broiler growth performance. Additionally, Pro and Post affected animal metabolism and leg health diet-dependently for some but not all investigated parameters. Our findings show that the effects of pro- and postbiotics on the growth performance and physiology of broilers can be dependent on diet composition and thus possibly other factors affecting diet characteristics.

Key words: broiler, dietary challenge, probiotic, postbiotic, mineral

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INTRODUCTION

Poultry production has continuously increased over the past decades and is today the first meat consumed and produced worldwide by humans (OECD and Food and Agriculture Organization of the United Nations, 2019). Feed ingredients availability is becoming more of an issue with high-quality ingredients becoming scarcer and more expensive. This encourages the use of alternative low-cost feed ingredients. However, the usage of unconventional ingredients is restricted, which is probably due to the presence of anti-nutritional compounds and lower digestibility of the feed nutrients compared to their conventional counterparts, resulting in reduced health, growth performance and welfare of the birds.

Additives, such as probiotics and postbiotics, have shown great potential to promote growth performance and health in broiler production (Humam et al., 2019; Jha et al., 2020). Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Jha et al., 2020), whereas postbiotics are defined as "a preparation of inanimate microorganisms and/or their components that confer health benefit to the host" (Vinderola et al., 2022).

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Several studies in poultry link pro- and postbiotics to effects on gut health and microbiota composition, fermentation, immunity (Jha et al., 2020), blood physiological parameters (e.g. related to health and nutrition) (Abd El-Hack et al., 2017; Humam et al., 2019) and bone metabolism (Liu et al., 2021). These effects are postulated to come from competitive exclusion and antagonism with other bacterial species, production and presence of beneficial metabolites (e.g., short chain fatty acids and vitamins) or interaction with immune and intestinal epithelial cells (Fathima et al., 2022). However, the broad spectrum of biological activities observed for pro- and postbiotics vary widely between microbial genera, species and strains, and between experiments (Pérez et al., 2016; Selim et al., 2022), with the origins and consequences of these variations remaining mostly unknown.

The lack of reproducible biological responses of pro- and postbiotics in broilers may partly be related to diet-dependent effects. This idea is supported by Abd El-Hack et al. (2017) who reported that, depending on diet, a *Bacillus subtilis* probiotic differently affected the production and characteristics of eggs as well as physiological parameters (blood serum albumin, triglycerides, cholesterol, Ca, P, and ammonia) in laying hens and suggested that a diet-induced performance depression allowed for a recovery effect of microbes-based additives. In mice, pigs and humans, dietary effects on the efficacy of probiotics are also reported (Liu et al., 2018; Larsen et al., 2023; Wastyk et al., 2023), providing extra evidence that diet composition could determine the phenotypic effects of pro- and postbiotics. For instance, under dietary challenging conditions, the potential impact of pro- and postbiotics on broilers growth can be expected to be greater because of the larger margin for improvement.

Diets containing specific ingredients, such as cereals and alternative protein sources can impair broiler performances (Beski et al., 2015; Polovinski-Horvatović, 2021). Particularly, nonstarch polysaccharides (**NSP**) found in cereals can impair nutrient digestibility, immunity and gut health, predispose poultry to the development of intestinal pathogens (Immerseel et al., 2004; Bindari and Gerber, 2022), favor footpad lesions and negatively affect bone quality (Bederska-Łojewska et al., 2017). Higher levels of NSP are present in rye (soluble) and barley (structural and soluble) compared to wheat and corn (Rath et al., 2000) and as such, without further supplementation, diets formulated with these ingredients could constitute a model for a dietary challenging condition for broiler growth and gut health (Mignon-Grasteau et al., 2020).

The present study aimed to assess the effect of 2 dietary conditions, designated standard diet (**SD**) and NSP-rich challenge diet (**CD**), on the efficacy of a *Lactobacilli*-based probiotic (**Pro**) and its derived postbiotic (**Post**) in broilers reared under production housing conditions. We hypothesized that 1) diet composition modulates the effect of Pro and Post on the growth performance and blood biochemical parameters of broilers and that 2) the effects of Pro and Post on growth performance are more pronounced under CD condition. Additionally, to gain more insight into the underlying effects, some parameters potentially affected by diet composition, probiotic and postbiotic were investigated, including bone characteristics as well as blood health and nutrition related parameters.

MATERIAL AND METHODS

Ethic Statement

The animal protocol for this research was approved by the Animal Welfare Committee of Zootest (Ploufragan, France) and complied with the guidelines in the European Union council directive 2010/63/EU for animal experiments. Animals were monitored daily, and handling and sampling took place under supervision of registered veterinarians.

Birds Housing and Management

A large batch of 1-day-old male Ross 308 broilers was purchased from a commercial hatchery (Galina Vendée, Daviet Ets, Essarts-en-bocage, France), with 1,368 chicks selected based on individual weights and distributed across 36 pens with 38 broilers each, so that all pens had a similar average chick body weight (**BW**) (~43.0 g) and distribution. Pens [1.90 × 1.25 × 0.8 m (L x W x H)] with wood shavings as floor covering were located along the wall of air entries on one side of a commercial, 1,200 m^2 Colorado type building. Water and feed per pen were provided ad libitum through nipple drinkers (5−6) and one 40 cm diameter Hung pan feeder (Josse, Montauban de Bretagne, France). The photoperiod was 24h light until d4 and then 20h from d5 to d42. Ambient temperature started at 32°C on d1 and, thereafter, gradually reduced in a linear fashion to 23°C on d22. Birds were daily inspected for lethargy, prostration and lameness and culled if found to be unhealthy. The total duration of the experiment was 42 d (d1−42).

Experimental Treatments

A completely randomized block design of 2 × 3 treatment groups with 2 diets and 3 additives was used. The 2 pelleted diets (SD and CD) were formulated based on commercial standards for nutrients levels for Ross 308 broilers and provided adequate and identical levels of all nutrients to the birds (Table 1), including apparent metabolizable energy, crude protein, essential amino acids and minerals. The SD was formulated to contain wheat, corn and soybean meal, whereas the CD contained in addition rye, barley and palm oil fat. The 2 diets were either unsupplemented (**Ctrl**) or supplemented with a commercial probiotic additive SORBIFLORE (in the present study referred as Pro) and its derived postbiotic additive METALAC (STI biotechnologie, Maen Roch, France) (here referred as Post).

Table 1. Ingredient and calculated composition including energy content of the standard and challenge starter, grower and finisher diet for broilers.

	Starter (0−11 d)		Grower (11−28 d)		Finisher (28−42 d)	
Composition	Standard	Challenge	Standard	Challenge	Standard	Challenge
Ingredient (% as is)						
Corn	29.7	18.1	35.8	18.3	42.8	28.6
Wheat	30.0	25.0	30.0	24.9	30.0	15.0
Barley	-	10.0	-	10.0	-	10.0
Rye	-	5.00	-	10.0	-	15.0
Soybean meal	33.5	33.6	28.3	28.5	21.9	23.4
Limestone	1.61	1.61	1.11	1.10	0.85	0.85
Mono calcium phosphate dihydrate	1.37	1.30	0.90	0.83	0.78	0.72
Sodium chloride 99%	0.25	0.25	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15
Soy oil	2.42	3.00	2.51	2.00	2.23	2.00
Palm oil	-	1.00	-	2.98	-	3.00
DL-methionine 99%	0.25	0.25	0.22	0.24	0.20	0.23
Lysine HCL 98%	0.16	0.15	0.18	0.17	0.25	0.21
L-threonine 98%	0.09	0.09	0.08	0.08	0.09	0.09
Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Calculated (% as is)						
Dry matter	87.7	88.1	87.6	88.0	87.4	88.1
Crude protein	22.0	22.0	20.0	20.0	17.6	17.7
Crude fat	4.44	5.72	4.76	6.71	4.71	7.08
Starch	38.6	36.1	42.5	38.7	46.8	41.9
Ash	6.50	6.55	5.26	5.30	4.54	4.67
Fibre[2]	12.7	13.9	12.3	13.7	11.5	13.0
Total NSP[2,3]	11.5	12.4	11.0	12.2	10.3	11.5
Soluble NSP[2]	2.59	3.02	2.41	2.93	2.11	2.66
Cellulose	2.68	2.97	2.56	2.85	2.42	2.69
Dig. Methionine	0.56	0.56	0.51	0.51	0.46	0.48
Dig. Methionine+cystine	0.91	0.91	0.84	0.84	0.76	0.76
Dig. Lysine	1.16	1.16	1.05	1.05	0.95	0.96
Dig. Threonine	0.78	0.78	0.70	0.71	0.64	0.64
Dig. Valine	0.90	0.90	0.82	0.82	0.72	0.73
Dig. Arginine	1.32	1.33	1.18	1.19	1.00	1.03
Calcium	0.96	0.96	0.68	0.67	0.54	0.54
Available phosphorous	0.46	0.46	0.34	0.34	0.30	0.30
Chlorine total	0.23	0.23	0.24	0.24	0.25	0.24
Sodium total	0.15	0.15	0.15	0.15	0.15	0.15
Apparent metabolizable energy (MJ/kg as is)	12.05	12.05	12.63	12.63	12.87	12.87

[1]Supplied per kg: 2,000,000 IU retinyl acetate, 500,000 IU cholecalciferol, 10 g DL-a-tocopherol, 300 mg menadione, 400 mg thiamine, 1,500 mg riboflavin, 700 mg pyridoxine-HCL, 4 mg cyanocobalamin, 7 g niacin, 2.4 g D-pantothenic acid, 92 g choline chloride, 200 mg folic acid, 40 mg biotin, 53 g $FeSO_4$·H2O, 9.6 g $CuSO_4$·$5H_2O$, 28 g MnO, 33 g $ZnSO_4$·H_2O, 240 mg KJ, 66 mg Na_2SeO_3.

[2]Values were calculated from levels in Knudsen (2014) and restricted to cereals and soybean meal.

[3]Nonstarch polysaccharide.

SORBIFLORE is the biomass resulting from the co-fermentation of a milk-based substrate by a mixture of L. rhamnosus CNCM-I-3698 and L. formosensis CNCM-I-3699, and METALAC is made from the heat-inactivated (high temperature short time) biomass of SORBIFLORE. Pro and Post biomasses were from the same production batch containing 3.1×10^9 colony forming units per gram of biomass and were dried as powder on carriers. Pro and Post biomasses were included in the diet at 50 g and 500 g biomass/t as is, respectively, from d1 to d11, and at 40 g and 400 g biomass/t as is, respectively, from d12 to d42.

Performance Data Collection

Individual BW and pen feed intake (**FI**) were recorded and feed conversion ratio (**FCR**) calculated on d11, 21, 28, and 35. Dead birds were daily collected and weighed to adjust pen FCR. Meat yield and quality were assessed on d42 from a subset of 63 broilers per treatment processed at a commercial slaughterhouse according to the French legislation. The broilers that either died during transportation or lost their individual tag were excluded from the meat measurements. The final dataset contained 59 to 63 broiler carcasses per treatment. On those, carcass, breast meat, and thighs were weighed, and wooden breast and white stripping were scored on a 3 point scale (0: absent, 1: intermediate, 2: severe) by one observer according to Sihvo et al. (2014) and Kuttappan et al. (2012), respectively.

Blood Sample Collection

For blood sampling, 4 broilers/pen (n = 24) were randomly selected on d11, wing tagged, and then sampled by wing vein puncture on d21 and 35, alternatively from right and left wing. In case of the death of a wing-tagged broiler, a new one was randomly selected in the same pen to keep 4 broilers sampled per pen. Puncture was compressed until bleeding stopped. Blood was collected in dry and heparin tubes (Greiner Bio-One, Les Ulis, France) for serum and plasma, respectively. Then, samples were

centrifuged (1,800 g; 5 min; room temperature) to recover the serum or plasma prior to storage at −20°C.

Plasma Biochemistry Parameter Evaluation

Blood plasma was analysed for their content in Ca, P, Mg, total protein, albumin, urea, uric acid (**UA**), creatinine, triglyceride, cholesterol, aspartate aminotransferase (**ASAT**), creatine phosphokinase (**CPK**), fructosamine, amylase activity, lipase activity and biliary acids with a ProVet analyser (Kitvia, Labarthe-Inard, France) according to the manufacturer's protocol. Description of the methods (including absorbance wavelengths) used are presented in Table S1. Globulins were determined as the difference between total protein and albumin.

Serum Coloration Evaluation

As a marker of gut integrity (Celi et al., 2019), carotenoid-based colorations of the bird's blood sera were assessed. Serum coloration was represented by obtaining the optical density values in every 10 nm wavelength interval within the 400 to 550 nm range, using a TriStar2S plate reader (Berthold Technologies, Wildbad, Germany) on 50 μL of blood serum samples in 96-well half-area microplates. Peak absorption values for carotenoids in blood are at 450 and 470 nm (Hamzic et al., 2015).

Leg Health, Strength, and Mineral Parameters

On d14 and 35, 3 and 4 broilers/pen were randomly selected, respectively, and euthanized by cervical dislocation for autopsy and tibia analyses. External soft tissues of the right leg were manually removed from the tibia using a scalpel. Then, the fresh tibias were weighed, and their strength (stiffness, maximum load force and breaking load force) determined by compression with a Synergie 200 (MTS, Eden Prairie, MN). Tibia were cradled on 2 support points with the crosshead approaching the middle of the bone at 100 mm/min until the bone broke. Load curves were plotted with TestWorkS software (MTS) following suitable calibration. Maximum load force was read at the peak of the curve with breaking load force corresponding to the point of bone breakage. Static stiffness was determined on the elastic deformation domain. Bone health was investigated at d35 with femoral head necrosis and non-adherent cartilage graded from both legs (0: absence, 1: presence in at least one leg) before the left tibia proximal end was longitudinally cut with a scalpel to grade tibia dyschondroplasia (**TD**) score (0: absence, 1: intermediate, 2: severe) and to measure the width of the articular cartilage, proliferation zone and mineralisation zone. Tibia dry matter (**DM**), ash, P, Ca, and Mg contents were measured at d35, in the same bones from which breaking strength was measured. Tibia were ground (Pulvérisette 11, Fritsch, Einersheim, Germany) and dried at 105°C for 21h before incineration at 550°C for 6h for determination of DM and ash content, respectively, both in triplicates. Ash samples from each individual were then pooled to measure ash P, Ca, and Mg content were determined according to theFR EN 15510 (2007) method (Dijkslag et al., 2019).

On d42, footpad lesions (**FPL**) were scored in all remaining birds (n=1050) with grade 0 allocated for no lesions, grade 1 for mild lesions and grade 2 for severe lesions.

Statistical Analyses

All analyses were performed with R version 4.0.3. Probability values $P < 0.05$ and $0.05 \leq P < 0.10$ were considered as significant and showing a trend, respectively. First, values deviating by more than 3 standard deviations from the mean were considered as outliers and excluded from the dataset.

Treatment effects on means were analyzed using the general linear model procedure and type 2 analysis of variance (library lme4 v1.1.31), with fixed effects of diet and additive, their interaction and a random block factor. For tibia weight and strength parameters as well as blood minerals, as birds had different BW, it was included as an additional co-variate. If the residuals were not normally distributed, a log or a boxcox transformation was applied with normality of residuals confirmed by Shapiro test. Variance homogeneity was assessed with Levene's test and, in case of heteroscedasticity, a White-Huber correction was applied. If residuals normality, checked by Shapiro test, could not be reached with any of the data transformation procedures (log and boxcox), a permutational analysis of variance was applied (function adonis2, library vegan v2.6-4) with 5,000 permutations and Euclidian distance as proposed by Anderson, 2017.

Treatment effects on femoral head necrosis and non-adherent cartilage were analyzed by logistic regression with glm and glmer functions (library lme4), respectively. Graded parameters were analyzed by ordinal logistic regression with the cumulative linked mixed model function for logistic regression. Logistic and ordered logistic models included a fixed diet and additive effects and their interaction as well as a random block factor, except for femoral head necrosis, which did not include the random block effect due to low occurrence.

For all statistical models, when an interaction effect was significant, intra-diet contrast (Pro vs. Ctrl and Post vs. Ctrl) and comparisons of controls (standard vs. challenge) analyses were performed. When a main additive effect was significant, inter-diet contrast analyses were performed to assess the overall effect of probiotic (Ctrl vs. Pro) and postbiotic (Ctrl vs. Post).

RESULTS

Growth Performances, Meat Yield, and Quality

The effect of the dietary treatments on broiler growth performance and FCR are presented in Table 2. On d35,

Table 2. Mean body weight (**BW**), daily feed intake (**dFI**) and feed conversion ratio (**FCR**) of 35 day-old male Ross 308 broilers fed a standard or challenge diet (**Ctrl**) supplemented with *Lactobacilli*-based probiotic (Pro) and postbiotic (Post) from d 1 onward.

	Diet[1]									
	Standard			Challenge			Pooled SEM	Main and interaction effects probability value		
Parameter	Ctrl	Pro	Post	Ctrl	Pro	Post		Diet	Additive	Diet × Additive
Body weight (g)										
d11	279	280	280	283	285	283	4.7	0.019	0.767	0.869
d21	848	832	849	854	865	857	19.8	0.017	0.835	0.179
d28	1,400	1,389	1,428	1,353	1,402	1,397	35.3	0.062	0.048	0.099
d35	2,033	2,002	2,075	1,936[+]	2,027[†]	1,997[†]	52.5	0.004	0.046	0.008
Daily feed intake (g)										
d1−11	26.2	26.5	26.5	27.4	27.6	26.6	0.9	<0.001	0.341	0.225
d12−21	80.4	78.7	80.8	83.7	85.2	84.4	3.1	<0.001	0.804	0.276
d22−28	123.3	125.3	126.9	122.6	128.4	129.2	6.5	0.390	0.047	0.649
d29−35	171.5	168.3	175.1	167.7	175.4	171.9	9.3	0.996	0.330	0.068
d1−35	89.8	89.2	91.2	89.8	93.4	92.1	10.2	0.062	0.206	0.134
Feed conversion ratio (g/g)										
d1−11	0.945	0.950	0.948	0.972	0.972	0.950	0.021	0.009	0.393	0.287
d12−21	1.430	1.442	1.455	1.489	1.494	1.498	0.037	<0.001	0.285	0.755
d22−28	1.586	1.579	1.539	1.725	1.673	1.692	0.050	<0.001	0.053	0.196
d29−35	1.901	1.931	1.899	2.031	1.966	2.012	0.108	0.002	0.887	0.387
d1−35	1.541	1.548	1.535	1.619	1.601	1.614	0.020	<0.001	0.717	0.148

[1]Body weight values are the mean of individual weights while values for daily feed intake and feed conversion ratio are the mean of 6 pen replicates per treatment.
[+]Mean of Ctrl is different from the Ctrl of the standard diet at $P < 0.05$.
[†]Mean of Pro or Post is different from its respective Ctrl at $P < 0.05$.

compared to broilers fed the Ctrl SD, broilers receiving the Ctrl CD diet had a lower BW (-4.8%). Moreover, broilers fed the CD had a higher 1-35d FCR (+4.5%) than birds fed the SD. This confirmed the challenging effect of CD. Supplementation with Pro or Post in the SD did not affect BW, daily FI and FCR on d35. However, when Pro or Post was added to CD, higher BW (+4.7% and +3.2%, respectively), but not different daily FI and FCR, were observed on d35, compared to their corresponding Ctrl birds. More specifically, CD decreased thighs yield (-1.4%), while increasing breast yield (+1.9%) relative to broiler BW (Table S2). An additive effect was observed for thighs/BW, but without significant contrast for Pro vs. Ctrl and a tendency for Post vs. Ctrl ($P = 0.079$) (Table S2). Treatments neither affected carcass yield nor meat quality (wooden breast and white stripping) parameters (Table S2). Overall mortality in the experiment was in a normal range of 4.8% (mean = 1.85 birds/pen on d42) and independent of the diets, additives and their interaction.

Leg Health and Growth

The effect of the dietary treatments on leg health was assessed by scoring TD, femoral head necrosis and

Table 3. Tibia dyschondroplasia, femoral head necrosis and non-adherent cartilage and footpad lesion score grading male Ross 308 broilers fed a standard or challenge diet (**Ctrl**) supplemented with *Lactobacilli*-based probiotic (**Pro**) and postbiotic (**Post**) from d 1 onward.

	Diet[1]								
	Standard			Challenge			Main and interaction effects probability value		
Parameter	Ctrl	Pro	Post	Ctrl	Pro	Post	Diet	Additive	Diet × Additive
Tibial dyschondroplasia grade (d35)									
0	16	19	22	8	9	12	<0.001	0.036	0.772
1	6	2	2	6	4	8			
2	2	3	-	10	8	4			
3	-	-	-	-	3	-			
Average	0.417	0.333	0.083	1.083	1.208	0.666			
Femoral head necrosis (d35)									
No	23	24	24	22	22	22	0.074	0.999	0.272
Yes	1	-	-	2	2	2			
Femoral non-adherent cartilage (d35)									
No	17	19	15	14	17	14	0.217	0.255	0.837
Yes	7	5	9	10	7	10			
Footpad lesion score (d42)									
0	82	96	81	86	94	105	0.042	0.014	0.028
1	42	60	56	44	57	46			
2	50	21	40	41	28	21			
Average	0.816	0.576[†]	0.768	0.737	0.631	0.512[†]			

[1]Count of animals per grade on all experimental birds for pododermatitis and n=24 for tibia dyschondroplasia, femoral head necrosis and non-adherent cartilage.
[†]Value of Pro or Post is different from its respective Ctrl at $P < 0.05$.

Table 4. Mean tibia head growth zone (d35), tibia weight and strength (d14 and 35) of male Ross 308 broilers fed a standard or challenge diet (**Ctrl**) supplemented with *Lactobacilli*-based probiotic (**Pro**) and postbiotic (**Post**) from d 1 onward.

	Diet[1]									
	Standard			Challenge				Main and interaction effects probability value[2]		
Parameter	Ctrl	Pro	Post	Ctrl	Pro	Post	Pooled SEM	Diet	Additive	Diet × Additive
Tibia head growth zone										
Width (mm)										
Articular cartilage	2.59	2.84	2.64	2.58	2.84	2.58	0.260	0.763	0.055	0.964
Growth plate	1.64	1.60	1.49	1.58	1.71	1.45	0.188	0.910	0.084	0.495
Mineralisation zone	3.28	3.76	3.48	4.95	4.41	4.71	0.679	<0.001	0.989	0.097
Total	7.55	8.20	7.61	9.03	8.99	8.74	0.797	<0.001	0.442	0.546
Relative width (as % of total)										
Articular cartilage	34.85	34.83	34.59	28.65	32.23	30.23	3.122	<0.001	0.422	0.353
Growth plate	22.10	19.88	19.67	17.50	19.26	17.11	2.244	<0.001	0.430	0.117
Mineralisation zone	43.05	45.29	45.74	53.85	48.51	52.66	4.231	<0.001	0.407	0.080
Tibia weight and strength										
d14										
Weight (g)	1.920	1.904	1.811	1.874	1.789	1.746	0.112	<0.001	0.065	0.858
Fracture force (N)	82.1	95.4†	78.9	90.6	82.4	77.9#	11.37	0.207	0.034	0.038
Maximum force (N)	98.1	101	89.5	96.9	92.5	92.5	8.74	0.056	0.155	0.234
Static Stiffness (N/m)	90.1	89.9	81.5	90.0	83.1	83.2	7.43	0.117	0.085	0.387
d35										
Weight (g)	9.46	10.61	10.34	10.02	10.21	10.19	0.715	0.200	0.238	0.219
Fracture force (N)	312.9	358.0	341.3	317.5	319.3	344.8	44.11	0.958	0.508	0.439
Maximum force (N)	330.9	364.4	348.3	335.3	343.9	355.7	37.70	0.577	0.793	0.656
Static stiffness (N/m)	214.4	232.5	239.6	196.7	205.5	219.0	23.63	0.005	0.046	0.982

[1]Tibia head growth zone values are the mean of 24 animals, excluding outliers. Bone weight and strength values are the mean of 18 and 24 animals at 14 and 35d, respectively, excluding outliers.
[2]*P*-values were body weight corrected in the ANOVA models.
†Mean of Pro or Post is different from its respective Ctrl at $P < 0.05$.
#Mean of Pro or Post is different from its respective Ctrl at $P < 0.10$.

femoral nonadherent cartilage on d35 and FPL on d42 (Table 3). Mean TD grade was higher in birds fed the CD compared to SD (+255%) and was lower in the Post groups compared to the 2 Ctrl groups (-50%; $P = 0.026$), whereas Pro treatment had no effect. Femoral head necrosis tended to be increased by CD but was not affected by additives. Femoral non-adherent cartilage occurrence was altered by neither diets, nor additives, nor their interaction and affected, on average, 33% of the birds. Mean FPL scores were not different between Ctrl SD and Ctrl CD. Supplementation of Pro and Post affected FPL sore in a diet-dependent manner. Specifically, mean FPL were reduced by Pro in the SD group (-29%) and Post in the CD group (-31%). Oppositely, no effect was observed for Pro under SD and for Post in SD.

Dietary treatments affected tibia head growth zones width on d35 (Table 4). Feeding CD increased mineralization zone (+35%) and total size (+15%), without affecting articular cartilage and growth plate width. The relative width of articular cartilage, growth plate and mineralization zone were affected by CD but not by additive treatments nor their interaction. No additive effect was observed on the absolute and relative tibia head growth zone width. Nonetheless, the trend for an additive effect on articular cartilage width yielded significant contrast analyses for Pro (+9.9%; $P = 0.032$), but not Post compared to Ctrl groups.

Tibia Weight and Strength

Dietary treatments and broiler age affected some of the tibia strength parameters (Table 4). On d14, mean tibia weight was lower in the CD group (-4.0%) and fracture force was higher when Pro was supplemented in the SD (+16.2%). The other tibia strength parameters were neither affected by diets, additives nor their interaction. On d35, tibia weight, fracture force and maximum force were not affected by treatments, but the static stiffness, however, was lower in broilers receiving CD (-9.5%). Furthermore, tibia stiffness was higher in Post (+11.6%; $P = 0.022$), but not in Pro-supplemented broilers, compared to the Ctrl groups.

Tibia and Plasma Minerals

The plasma concentration of certain minerals was affected by diet and additive (Table 5). Plasma Ca/P ratio decreased with CD at d21 through a decreased Ca level, whereas at d35, it increased through a decreased P level. Pro increased plasma P and Ca on d21 (+6.2 and +7.4%, respectively) and d35 (+6.6 and +6.2%, respectively), whereas Ca/P ratio and Mg levels were not affected at both ages. Post treatment increased plasma Mg by 11.3% at d21 and did not affect the other parameters.

Diets, but not additives, affected tibia DM and ash at d35 (Table 5). Broilers fed the CD had, relative to tibia fresh weight, a lower tibia DM and ash content. However, tibia ash content relative to tibia DM was not affected. Tibia ash Ca and P content were affected by additives in a diet-dependent fashion only (Table 5). Intra-diet contrasts showed Ca and P to be reduced by Pro in broilers fed SD (3.2 and 2.7%), without other significant contrasts ($P > 0.1$). Tibia ash Mg content

Table 5. Mean calcium (**Ca**), phosphorus (**P**) and magnesium (**Mg**) concentration in tibia on d35 and plasma on d21 and 35 of male Ross 308 broilers fed a standard or challenge diet (Ctrl) supplemented with *Lactobacilli*-based probiotic (**Pro**) and postbiotic (**Post**) from d 1 onward.

	Diet[1]							Probability value[2]				
	Standard			Challenge			Pooled SEM	Main and interaction effects			Contrasts	
Parameter	Ctrl	Pro	Post	Ctrl	Pro	Post		Diet	Additive	Diet × Additive	Pro vs. Ctrl	Post vs. Ctrl
d21												
Plasma (mM)												
Ca	2.58	2.74	2.65	2.44	2.59	2.40	0.147	<0.001	0.014	0.682	0.013	0.866
P	2.10	2.26	2.17	2.10	2.25	2.12	0.143	0.413	0.021	0.930	0.007	0.542
Ca/P	1.25	1.23	1.23	1.18	1.18	1.16	0.094	0.063	0.883	0.957	-	-
Mg	1.00	0.97	1.10	1.04	0.97	1.17	0.168	0.372	0.010	0.771	0.409	0.037
d35												
Plasma (mM)												
Ca	2.41	2.58	2.52	2.45	2.60	2.40	0.153	0.618	0.027	0.410	0.013	0.756
P	2.00	2.14	2.00	1.89	1.99	1.80	0.149	0.001	0.012	0.839	0.042	0.516
Ca/P	1.26	1.22	1.26	1.31	1.33	1.34	0.080	0.001	0.726	0.917	-	-
Mg	1.09	1.12	1.21	1.16	1.02	1.08	0.195	0.513	0.455	0.314	-	-
Tibia												
DM (%FW)[3]	46.98	47.21	48.23	43.23	44.06	44.55	1.910	<0.001	0.285	0.832	-	-
Ash (%FW)[3]	23.71	23.89	24.29	21.86	22.44	22.75	1.193	<0.001	0.371	0.754	-	-
Ash (%DM)[4]	50.48	50.60	50.39	50.41	50.82	51.01	1.180	0.351	0.946	0.616	-	-
Ca (% Ash)	34.98	33.84†	35.32	34.47	34.76	34.17	0.776	0.286	0.189	<0.001	-	-
P (% Ash)	17.80	17.32†	17.95	17.72	17.75	17.54	0.362	0.869	0.189	0.007	-	-
Ca/P	1.966	1.954	1.968	1.956	1.957	1.948	0.018	0.064	0.391	0.061	-	-
Mg (% Ash)	0.775	0.769	0.771	0.802	0.777	0.804	0.030	0.021	0.354	0.539	-	-

[1]Values are mean of 24 animals per treatment, excluding outliers.
[2]*P*-values were body weight corrected in the ANOVA/PERMANOVA models.
[3]Mean expressed as percentage relative to fresh tibia weight.
[4]Mean expressed as percentage relative to tibia dry matter.
†Mean of Pro is different from its respective Ctrl at $P < 0.05$.

showed only a diet effect, with Mg level higher in broilers fed CD compared to SD.

Plasma Biochemical Parameters for Nutritional Metabolism

Diet effects on plasma parameters depended on the age of the broiler (Table 6). On d21, the CD increased plasma cholesterol (+9.1%) and decreased lipase activity (-25.6%), whereas these parameters were not affected at d35. Plasma bile acids where higher in the Ctrl CD group compared to the Ctrl SD group with +67.0 and +25.3% at d21 and 35, respectively. At both ages, the CD did not affect plasma fructosamine (as a measure of total glycated serum proteins), uric acid, triglycerides, triglyceride to cholesterol ratio and amylase activity.

Pro affected several plasma parameters, with effects depending on diet and age of the birds (Table 6). Pro increased plasma uric acid on d21 (+15.5%) and 35 (+21.0%). On d35, Pro increased plasma triglyceride (+19.5%) but had no effect on cholesterol and consequently increased triglyceride to cholesterol ratio (+17.0%), whereas Pro had no effects on those parameters on d21. On d35, the tendency for an additive effect on fructosamine concentration led to a significant contrast for Pro vs. Ctrl by +3.5%. Plasma lipase activity was not affected by Pro on d35 but on d21, Pro decreased lipase activity in SD (-22.2%) and increased it in CD (+20.7%). Pro increased plasma bile acids in SD on d21 (+46.2%) and 35 (+39.8%), but decreased plasma bile acids in CD on d35 (-25.8%) and had no effect on d21.

Post had limited effects on plasma parameters (Table 6). Post increased plasma bile acids in SD at both ages (+73.5% and +29.6% at d21 and 35, respectively), but in CD, Post tended to decrease bile acids at d21 (-24.5%; $P = 0.085$) and increased them significantly on d35 (+27.3%). Post did not affect plasma lipase activity at d35, whereas at d21, Post decreased plasma lipase activity in SD by -30.0% but increased it by +52.3% in CD. The tendencies for an additive effect on cholesterol concentration on d21 and fructosamine on d35 yielded, for Post effect, contrast *P*-values of 0.046 and 0.053, respectively.

Blood Biochemical Parameters Related to Health and Nutritional Metabolism

As shown in Table 6, at d21, the CD and Pro treatment significantly increased plasma total protein concentration by 6.6% and 4.5%, respectively, whereas on d35, this parameter was not affected by treatments. The effect of Pro on plasma total protein concentration at d21 was higher in CD compared to SD as the tendency for an interaction effect led to significant intra-diet contrasts for a Pro effect only in CD ($P = 0.013$). At both ages, the carotin-based serum optic density at 450 and 470 nm was not affected by diet, additive and their interaction and averaged 0.524 ± 0.080 and 0.471 ± 0.067, respectively on d21 and 0.634 ± 0.087 and 0.578 ± 0.074, respectively on d35.

Table 6. Mean plasma concentration and activity for health- and nutrition-related biochemical parameters on d21 and 35 of male Ross 308 broilers fed a standard or challenge diet (**Ctrl**) supplemented with *Lactobacilli*-based probiotic (**Pro**) and postbiotic (**Post**) from d 1 onward.

	Diet[1]							Probability value[2]				
	Standard			Challenge			Pooled SEM	Main and interaction effects			Contrasts	
Parameter	Ctrl	Pro	Post	Ctrl	Pro	Post		Diet	Additive	Diet × Additive	Pro vs. Ctrl	Post vs. Ctrl
d21												
Nutrition												
Fructosamine (μmol/L)	241	254	254	260	251	256	13	0.258	0.756	0.115	-	-
Uric acid (μmol/L)	415	493	436	468	527	469	67	0.101	0.039	0.991	0.025	0.956
Triglyceride (mmol/L)	0.60	0.65	0.70	0.70	0.83	0.67	0.13	0.127	0.282	0.126	-	-
Cholesterol (mmol/L)	3.58	3.39	3.27	3.63	3.82	3.53	0.24	0.002	0.066	0.150	-	-
Triglyceride/cholesterol	0.18	0.19	0.21	0.20	0.22	0.19	0.04	0.737	0.402	0.447	-	-
Bile acids (μmol/L)	5.06	7.40†	8.78†	8.45+	8.21	6.38#	1.88	0.481	0.242	0.002	-	-
Lipase (U/L)	23.4	18.2†	16.4†	17.4+	21.0†	26.5†	3.2	0.014	0.460	<0.001	-	-
Amylase (U/L)	1800	1308	1861	1613	1745	1919	391	0.320	0.135	0.182	-	-
Health and nutrition												
Total protein (g/L)	31.7	32.0	31.4	32.5	35.1	31.3	1.8	0.011	0.008	0.072	0.045	0.359
Health												
Albumin (g/L)	13.9	13.9	13.9	14.4	15.4	14.0	0.8	0.002	0.038	0.057	0.074	0.587
Globulins (g/L)	17.9	18.1	17.5	18.1	19.7	17.3	1.4	0.082	0.024	0.218	0.063	0.451
Albumine/Globuline	0.79	0.78	0.80	0.79	0.79	0.80	0.05	0.861	0.784	0.623	-	-
CPK[3] (U/L)	2478	2067	2342	3374	2472	3088	749	0.003	0.545	0.986	-	-
ASAT[4] (U/L)	241	241	246	256	252	264	17	0.006	0.397	0.871	-	-
Creatinine (μmol/L)	6.41	7.53	7.95	6.33	7.74	4.89	2.27	0.115	0.338	0.130	-	-
Urea (mmol/L)	0.31	0.41	0.41	0.43	0.42	0.49	0.13	0.056	0.300	0.286	-	-
d35												
Nutrition												
Fructosamine (μmol/L)	299	306	312	295	309	301	15	0.341	0.094	0.462	-	-
Uric acid (μmol/L)	259	342	299	307	343	301	61	0.285	0.009	0.313	0.005	0.758
Triglyceride (mmol/L)	0.98	1.24	1.09	1.02	1.15	0.97	0.16	0.185	0.003	0.378	0.001	0.642
Cholesterol (mmol/L)	3.86	3.88	3.95	3.80	3.83	3.92	0.23	0.472	0.385	0.963	-	-
Triglyceride/cholesterol	0.26	0.32	0.28	0.27	0.30	0.24	0.04	0.193	0.001	0.168	0.002	0.799
Bile acids (μmol/L)	5.50	7.69†	7.13†	6.89*	5.11†	8.77†	1.30	0.979	0.001	<0.001	-	-
Lipase (U/L)	17.7	18.0	18.9	17.0	16.8	16.7	2.8	0.573	0.730	0.709	-	-
Amylase (U/L)	1066	948	929	1189	1140	1072	260	0.356	0.704	0.898	-	-
Health and nutrition												
Total protein (g/L)	29.9	31.6	30.9	31.3	31.6	31.0	1.9	0.554	0.272	0.667	-	-
Health												
Albumine (g/L)	11.9	12.3	12.4	12.2	12.6	11.9	0.8	0.946	0.388	0.418	-	-
Globulins (g/L)	18.0	19.4	18.6	18.9	19.1	19.1	1.3	0.495	0.208	0.488	-	-
Albumine/Globuline	0.67	0.64	0.67	0.65	0.67	0.63	0.04	0.375	0.818	0.061	-	-
CPK (U/L)	6341	7838	6722	6488	7115	7711	1,797	0.467	0.281	0.570	-	-
ASAT (U/L)	296	302	276	318	293	325	35	0.063	0.867	0.236	-	-
Creatinine (μmol/L)	4.92	5.07	5.83	4.18	6.47	3.97	2.28	0.316	0.170	0.230	-	-
Urea (mmol/L)	0.29	0.27	0.27	0.25	0.29	0.25	0.08	0.601	0.781	0.680	-	-

[1]Values are mean of 24 animals per treatment, excluding outliers.
[2]*P*-values were corrected for broiler body weight as cofactor.
[3]Creatinine phosphokinase.
[4]Aspartate aminotransferase.
†Mean of Pro or Post is different from its respective Ctrl at $P < 0.05$.
#Mean of Pro or Post is different from its respective Ctrl at $P < 0.10$.
+Mean of Ctrl challenge diet is different from the Ctrl of the standard diet at $P < 0.05$.
*Mean of Ctrl challenge diet is different from the Ctrl of the standard diet at $P < 0.10$.

Plasma Biochemical Biomarkers for Health Status

Diet and additive effects on plasma health biomarker parameters were age and to some extent diet-dependent (Table 6). On d21, CD significantly increased plasma CPK (+29.7%), total protein (+6.6%) and albumin (+7.2%) and tended to increase globulin (+6.0%) concentration, without affecting albumin to globulin ratio. At this age, Pro increased total protein (+4.5%) and tended to increase albumin (+3.5%) and globulin (+5.0%) levels. The effect on blood albumin concentration was higher in CD compared to SD as the tendency for an interaction effect led to significant intra-diet contrasts for a Pro effect only in CD ($P = 0.018$). Post had no effect on those parameters on d21. On d35, plasma albumin, globulins, albumin/globulins, CPK, ASAT creatinine and urea concentration were not affected by diet, additive and their interaction (Table 6).

DISCUSSION

This study aimed to assess the effects of 2 dietary conditions (i.e. SD and CD) on the efficacy of *Lactobacilli*-based Pro and Post supplementation on the growth performance in broilers and on their mineral metabolism, leg health and blood physiological parameters. We

hypothesized that 1) diet composition modulates the effect of Pro and Post on broiler growth performance and blood physiological parameters, and that 2) the effects of Pro and Post on growth performance are greater under dietary challenge conditions. To this end, we used an NSP-rich (i.e., rye and barley-based) diet to challenge the animals. As expected, feeding broilers with CD resulted in lower BW, greater FCR and impaired tibia health and strength, compared to SD. Strikingly, supplementation with Pro or Post alleviated the deleterious effect of CD on BW, while it did not affect growth when supplied in SD. As discussed hereafter, this was associated with additional diet-dependent and independent effects on some of the other measured parameters leading to the acceptance of both hypotheses.

Our results show that diet composition modulated the effects of the tested *Lactobacilli*-based additives, with the challenging dietary condition showing a Pro and Post recovery effect on broiler growth (Table 2). On d35, the CD depressed broiler growth without affecting FI and, therefore, increased FCR, whereas at younger age, when rye inclusion was lower (d11 and 21), broilers fed the CD had a greater BW, FI and FCR. Interestingly, a low level of rye (5%) at young age (<11 d) seems to improve growth through increased FI, which did not occur at older age possibly because of limiting FI capacity. Since the diets were formulated to contain identical levels of apparent metabolizable energy and nutrients (e.g. proteins, amino acids, minerals), this indicates that the growth depression evoked by CD at higher age was most likely attributed to the incremental inclusion of rye and a reduction of the bioavailability of nutrients for growth. This reduction can result from a lower digestibility and absorption of the nutrients in the diet (i.e., bioaccessibility), but may also originate from a decreased post-absorptive utilization (i.e., bioactivity). This CD-induced growth depression effect was negated by Pro and Post additives, as shown by the greater BW at d35. In contrast, in SD, Pro and Post showed no effects on BW, FI and FCR. These observations indicate that some of the deleterious effects of CD, inducing the growth depression, were counteracted by Pro and Post treatments.

The CD-induced growth depression in the broilers likely originated from the inclusion of rye and barley, which contain higher concentrations of total NSP and a higher proportion of soluble NSP, compared to wheat and corn (Knudsen, 2014). These polysaccharides and particularly soluble NSP increase digesta water holding capacity and viscosity (Bederska-Łojewska et al., 2017). High viscosity decreases free diffusion of endogenous enzymes in the chyme and lowers the interaction with the brush border, thereby, potentially reducing nutrient digestion and absorption. In the literature, rye and barley have been shown to impair performance, increase digesta viscosity and mean retention time, promoting an environment for growth of harmful bacteria (Lazaro et al., 2003; Mehrabadi and Jamshidi, 2019). In addition, in broilers, rye inclusion at 5 and 10% increased digesta viscosity, increased jejunum cell turnover, and activated mechanisms for pathogen eradication, including the complement and coagulation pathways which are part of the innate immune system (van Krimpen et al., 2017). These processes require nutrients and reduce their availability for use in growth processes. Aside from soluble NSP, the insoluble NSP (structural) present in higher proportion in barley compared to wheat and corn, could have also contributed to the deleterious effect of CD by acting as a physical barrier to digestive enzymes, increase of endogenous losses (Knudsen, 2014; Nguyen et al., 2021). An increase in insoluble NSP can also have beneficial effects on broilers (Nguyen et al., 2021), but since CD depressed growth performance, the deleterious effect of the NSP in CD-fed broilers may have been greater than the beneficial ones. The positive contribution of Pro and Post on growth with CD intake may be ascribed to the counteracting of NSP-induced deleterious effects through improved digestibility, gut microbiota composition and host immunity, effects that are commonly reported for probiotics (Fesseha, 2019; Abd El-Hack et al., 2020) and postbiotics (Piqué et al., 2019).

Next, we observed a diet-dependent effect of Pro and Post on FPL. Pro and Post reduced mean FPL score, with a more pronounced effect of Pro under SD and Post under CD (Table 3). Footpad lesions are necrotic lesions caused by skin inflammation (Tellez et al., 2014). They are promoted by high litter and excreta humidity and a high excretion of undigested protein (Swiatkiewicz et al., 2017), which are both promoted by poor gut health (Hermans et al., 2006). Some of the latter parameters could have been improved by Pro and Post.

Bone growth and strength in the broilers was affected by dietary treatments, CD and Post (Tables 3, 4, and 5). Post treatment improved bone formation in both diets as shown by an increased tibia stiffness and lower incidence of TD at d35. The CD impaired tibia mineralization as evidenced by lower dry matter and ash, altered Ca/P and Mg content, higher TD score and lower stiffness. Accordingly to our results, feeding broilers with a rye-based diet negatively affected bone quality in broilers and this was associated with the malabsorption of fat-soluble vitamins and minerals (Tellez et al., 2014). Bone strength is related to its physical, architectural and material properties (Rath et al., 2000). Tibia mass, ash, Ca, P, and Mg content were not affected by Post, therefore, the increased stiffness is more likely to originate from tibia shape, collagen fiber orientation, other minerals or specific molecules in the matrix. Accordingly, TD score was negatively correlated with tibia stiffness ($P < 0.001$). The mechanism(s) underlying TD remain poorly understood but has been associated with a deficiency in nutrients essential for bone growth, and oxidative stress and inflammation in the growth plate (Dong et al., 2022). Post unlike Pro evoked an increase in blood Mg at d21 compared to their corresponding Ctrl groups likely because of the presence of sepiolite ($Mg_4Si_6O_{15}(OH)_2$) in the carrier. The role of Mg in bone formation is barely studied in chickens (Shastak and Rodehutscord, 2015), but in humans, Mg deficiency reduces bone stiffness (Castiglioni et al., 2013).

However, no difference in tibia Mg content was observed. Thus, dietary and blood Mg levels could not explain the increased tibia strength and decreased TD observed with Post.

Surprisingly, Pro increased the thickness of the tibia head articular cartilage by 9.9% on average, independently of the diet. Cartilage homeostasis between synthesis and degradation is maintained and controlled by chondrocytes. Recent reviews support the notion that some exogenous molecules can support cartilage growth and extracellular matrix synthesis (Li et al., 2020) and that the microbiota may have a role in cartilage development and injury (Hao et al., 2021). In other species, probiotics were found to slowdown induced osteoarthritis (cartilage breakdown) in association with reduced local inflammation (Sophocleous et al., 2020), but, to our knowledge, this is the first time that a probiotic is reported to impact cartilage growth in chickens.

Notably, we observed that the effects of Pro and Post were different between ages and diets on plasma lipase activity and bile acids, which suggests a diet x additive x age interaction effect. Lipase activity in the blood serum and pancreas are strongly correlated ($r = 0.96$) (Vertiprakhov et al., 2018). The Pro and Post additives increased blood plasma lipase activity on d21 in CD but decreased it in SD, whereas no effects were observed on d35. The CD decreased plasma lipase activity by 26% despite that this diet contained more fat, opposing the results of Dror et al. (1976). Plasma bile acids were strongly affected by Pro and Post. Both additives increased plasma bile acids in SD on both d21 and d35, whereas in CD, Pro had no effect on d21 and a lowering effect on d35. Post lowered plasma bile acids on d21 while increasing them on d35. Blood bile acid concentrations are the result of complex physiological regulation mechanisms (Arshad et al. 2021). Bile acids are synthetized in the liver *de novo* from cholesterol, concentrated in the gallbladder and expelled in the gut to promote fat digestion. Most of the bile acids are reabsorbed by enterocytes (~95%), thereby minimalizing their losses in the faces. Bile acids are also biotransformed into secondary bile acids by gut microbiota (Arshad et al. 2021). Thus, a diet-dependent modulation of the gut microbiota, of its activity or both could have mediated Pro and Post effects on plasma bile acids.

Contrary to CD, Pro, and Post had neither deleterious nor beneficial effects on blood plasma health parameters in the broilers. Plasma proteins were affected by CD and Pro on d21 only but remained within normal range (Filipović et al., 2007; Piotrowska et al., 2011). Similar to our findings, other studies reported no effect of pro- and postbiotic on serum or plasma total protein and albumin (Alkhalf et al., 2010; Hatab et al., 2016; Wu et al., 2019; Hussein et al., 2020; Zhu et al., 2020), whereas Yazhini et al. (2018) reported an increase in plasma total protein and albumin concentration with postbiotic supplementation in broilers. Feeding CD appeared to have a temporary effect on plasma CPK and ASAT levels, since both metabolites were increased on d21, but not on d35. Blood ASAT is a sensitive but non-specific indicator for liver and muscle damage (Amaral et al., 2017), while CPK in birds is a sensitive and more specific indicator of muscle damage (Lumeij, 2008). Furthermore, an increase in blood urea and creatinine levels may indicate renal disfunction (Valchev et al., 2014). Previous studies did not report consistent effects of pro- and postbiotics on blood ASAT, CPK, creatinine and urea concentration (Biswas et al., 2018; Wu et al., 2019; Hussein et al., 2020; Zhu et al., 2020), possibly because of different genera, strains and contexts. Hussein et al. (2020) reported no difference on serum ASAT level in broilers supplemented with a *B. subtilis* probiotic. Biswas et al. (2018) reported that a *L. acidophilus* probiotic increased serum ASAT and creatinine concentration. Wu et al. (2019) reported decreased serum CPK after *L. plantarum* probiotic supplementation. Contrary to Pro, very few studies investigated Post effect on broiler biochemical parameters. Zhu et al. (2020) reported that plasma ASAT was not affected, but creatinine concentration decreased in postbiotic supplemented broilers with an age-related effect. Results on blood serum optic density at 450 and 470 nm indicates that carotenoids were not affected by dietary Pro and Post. Since broilers cannot synthetize carotenoids, they originate only from gut absorption and can serve as a marker of gut integrity (Celi et al., 2019). Beside their use as gut integrity and fat digestibility marker, it has to be mentioned that carotenoids are also implied in vitamins synthesis, immune response and control of oxidative stress, which can influence their concentration in blood (Figuerola et al., 2014).

Under both dietary conditions, Pro increased plasma Ca, P, uric acid, triglyceride and fructosamine concentrations. The higher plasma Ca and P concentrations can be the result of increased absorption and reabsorption of these minerals in the intestine and kidney, respectively, or by lower bone accretion (Proszkowiec-Weglarz and Angel, 2013). Accordingly, previous studies reported that probiotics could increase intestinal Ca and P absorption in chickens and pigs (Pérez et al., 2016; Selim et al., 2022). The increase in blood plasma uric acid with Pro may be a result of an improvement in amino acids absorption. A lower deposition of amino acids is less likely since meat yield was not reduced by Pro and since blood creatinine, a marker of muscle mass and kidney function, was not affected (Piotrowska et al., 2011). Our results on plasma uric acid are not in line with those of Biswas et al. (2018). Surprisingly, no studies have reported an increase in blood triglycerides with probiotic supplementation, while many showed opposite results (Kalavathy et al., 2003; Ashayerizadeh et al., 2011; Yazhini et al., 2018). These authors explained their results by increased intestinal lactic acid bacteria, which is known to lower triglyceride absorption through deconjugating bile salts and subsequently lowering blood triglycerides.

The observation of increased plasma fructosamine concentration with Pro on d35 may indicate a higher blood glucose level in relation with elevated amino acid catabolism and a greater absorption of dietary starch.

Indeed, fructosamines result from blood protein glycosylation due to an increase of glycemia (Klandorf et al., 1995), while blood glucose is modulated by the absorption of glucose from dietary starch, de-novo synthesis from amino-acids and endocrine system (Braun and Sweazea, 2008).. Circulating glucose is used for lipogenesis, increasing blood triglycerides (Zaefarian et al., 2019), which was also observed in the present study. Lipogenesis also occurs from absorbed dietary fat (Zaefarian et al., 2019), but fat digestibility may not have been affected since Pro did not affect serum optic density at 450 and 470 nm. The latter wavelengths correspond to the carotenoids concentration (Hamzic et al., 2015), which can be used as an indirect marker of fat digestibility (Mignon-Grasteau et al., 2020). Overall, the data suggest that Pro has improved nutrient digestibility in both dietary conditions.

The current study supports the idea that Pro and Post may have had a beneficial effect on some of the deleterious effects of CD, expected to result from the higher NSP and particularly soluble NSP level in this diet. It must be pointed out that the deleterious effect of NSP can be counteracted by NSPase (Polovinski-Horvatović, 2021). Thus, NSPase addition in CD would probably have affected Pro and Post effects and particularly lowered their effects on growth.

The results of the present study highlight that diet composition can affect probiotic and postbiotic effects in broilers and thus that diet formulas must be considered to evaluate the effects of microbes-based additives. We provide a first indication of a diet and microbe-based additive interaction on growth, health status and metabolism in broilers. Further studies are required to confirm our observations and to test whether probiotic and postbiotic could also interact with other feed ingredients or feed additives affecting diet characteristics.

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DISCLOSURES

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SUPPLEMENTARY MATERIALS

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REFERENCES

Abd El-Hack, M. E., M. T. El-Saadony, M. E. Shafi, S. Y. A. Qattan, G. E. Batiha, A. F. Khafaga, A.-M. E. Abdel-Moneim, and M. Alagawany. 2020. Probiotics in poultry feed: a comprehensive review. J. Anim. Physiol. Anim. Nutr. 104:1835–1850.

Abd El-Hack, M. E., S. A. Mahgoub, M. Alagawany, and E. A. Ashour. 2017. Improving productive performance and mitigating harmful emissions from laying hen excreta via feeding on graded levels of corn DDGS with or without Bacillus subtilis probiotic. J. Anim. Physiol. Anim. Nutr. 101:904–913.

Alkhalf, A., M. Alhaj, and I. Al-homidan. 2010. Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. Saudi J. Biol. Sci. 17:219–225.

Amaral, P. C., C. Zimermann, L. R. Santos, M. Noro, M. D. Prá, F. Pilotto, L. B. Rodrigues, and E. L. Dickel. 2017. Evaluation of physiological parameters of broilers with dorsal cranial myopathy. Braz. J. Poult. Sci. 19:69–74.

Anderson, M. J. 2017. Permutational multivariate analysis of variance (PERMANOVA). Pages 1−15 in Wiley StatsRef: Statistics Reference Online. N. Balakrishnan, T. Colton, B. Everitt, W. Piegorsch, F. Ruggeri and J. L. Teugels, eds. John Wiley & Sons, Ltd.

Arshad, M. A., S. A. Faiz-ul-Hassan, Bhatti, M. S. Rehman, W. Yousaf, G. Younus, O. Sizmaz, and M. Q. Bilal. 2021. Supplementation of bile acids and lipase in broiler diets for better nutrient utilization and performance: potential effects and future implications – a review. Ann. Anim. Sci. 21:757–787.

Ashayerizadeh, A., N. Dabiri, K. Mirzadeh, and M. Ghorbani. 2011. Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. J. Cell Anim. Biol. 5:152–156.

Bederska-Łojewska, D., S. Świątkiewicz, A. Arczewska-Włosek, and T. Schwarz. 2017. Rye non-starch polysaccharides: their impact on poultry intestinal physiology, nutrients digestibility and performance indices – a review. Ann. Anim. Sci. 17:351–369.

Beski, S. S. M., R. A. Swick, and P. A. Iji. 2015. Specialized protein products in broiler chicken nutrition: a review. Anim. Nutr. 1:47–53.

Bindari, Y. R., and P. F. Gerber. 2022. Centennial Review: Factors affecting the chicken gastrointestinal microbial composition and their association with gut health and productive performance. Poult. Sci. 101:101612.

Biswas, A., N. Junaid, M. Kumawat, S. Qureshi, and A. B. Mandal. 2018. Influence of dietary supplementation of probiotics on intestinal histo-morphometry, blood chemistry and gut health status of broiler chickens. S. Afr. J. Anim. Sci. 48:968–976.

Braun, E. J., and K. L. Sweazea. 2008. Glucose regulation in birds. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 151:1–9.

Castiglioni, S., A. Cazzaniga, W. Albisetti, and J. A. M. Maier. 2013. Magnesium and osteoporosis: current state of knowledge and future research directions. Nutrients 5:3022–3033.

Celi, P., V. Verlhac, E. Pérez Calvo, J. Schmeisser, and A.-M. Kluenter. 2019. Biomarkers of gastrointestinal functionality in animal nutrition and health. Anim. Feed Sci. Technol., SI: Intestinal Health 250:9–31.

Dijkslag, M. A., M. L. Elling-Staats, Y. Yen, L. M. Marchal, and R. P. Kwakkel. 2019. The effects of coarse and wet feeding on performance parameters, gastrointestinal tract and tibia traits, and digesta phytase activity in egg-type pullets, either fed a low or moderate phosphorus diet. Poult. Sci. 98:4729–4744.

Dong, P.-F., C. Jin, C.-Y. Lian, L. Wang, and Z.-Y. Wang. 2022. Enhanced extracellular matrix degradation in growth plate contributes to manganese deficiency-induced tibial dyschondroplasia in broiler chicks. Biol. Trace Elem. Res. 200:3326–3335.

Dror, Y., A. Shamgar, and P. Budowski. 1976. Effect of dietary fat on pancreatic lipase activity in chicken. Int. J. Vitam. Nutr. Res. 46:83–86.

Fathima, S., R. Shanmugasundaram, D. Adams, and R. K. Selvaraj. 2022. Gastrointestinal microbiota and their manipulation for improved growth and performance in chickens. Foods 11 11:1401.

Fesseha, H. 2019. Probiotics and its potential role in poultry production: a review. Vet. Med. Open J. 4:69–76.

Figuerola, J., G. López, and R. Soriguer. 2014. Plasma carotenoid levels in passerines are related to infection by (some) parasites. Front. Ecol. Evol. 2.

Filipović, N., Z. Stojević, S. Milinković-Tur, B. B. Ljubić, and M. Zdelar-Tuk. 2007. Changes in concentration and fractions of blood serum proteins of chickens during fattening. Vet. Arh. 77:319–326.

FR EN 15510, 2007. Aliments des animaux - Méthodes d'échantillonnage et d'analyse - Détermination des teneurs en calcium, sodium, phosphore, magnésium, potassium, fer, zinc, cuivre, manganèse, cobalt, molybdène et plomb par ICP-AES, AFNOR. ed.

Hamzic, E., B. Bed'Hom, H. Juin, R. Hawken, M. S. Abrahamsen, J. M. Elsen, B. Servin, M. H. Pinard-van der Laan, and O. Demeure. 2015. Large-scale investigation of the parameters in response to Eimeria maxima challenge in broilers. J. Anim. Sci. 93:1830–1840.

Hao, X., X. Shang, J. Liu, R. Chi, J. Zhang, and T. Xu. 2021. The gut microbiota in osteoarthritis: where do we stand and what can we do? Arthritis Res. Ther. 23:42.

Hatab, M. H., M. A. Elsayed, and N. S. Ibrahim. 2016. Effect of some biological supplementation on productive performance, physiological and immunological response of layer chicks. J. Radiat. Res. Appl. Sci. 9:185–192 Special issue on: "Current Research and Future Trends in Biotechnology for a Healthy and Green Word".

Hermans, P. G., D. Fradkin, I. B. Muchnik, and K. L. Organ. 2006. Prevalence of wet litter and the associated risk factors in broiler flocks in the United Kingdom. Vet. Rec. 158:615–622.

Humam, A. M., T. C. Loh, H. L. Foo, A. A. Samsudin, N. M. Mustapha, I. Zulkifli, and W. I. Izuddin. 2019. Effects of feeding different postbiotics produced by Lactobacillus plantarum on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. Animals 9:644.

Hussein, E. O. S., S. H. Ahmed, A. M. Abudabos, M. R. Aljumaah, M. M. Alkhlulaifi, M. A. Nassan, G. M. Suliman, M. A. E. Naiel, and A. A. Swelum. 2020. Effect of antibiotic, phytobiotic and probiotic supplementation on growth, blood indices and intestine health in broiler chicks challenged with clostridium perfringens. Animals 10:507.

Immerseel, F. V., J. D. Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. Clostridium perfringens in poultry: an emerging threat for animal and public health. Avian Pathol 33:537–549.

Jha, R., R. Das, S. Oak, and P. Mishra. 2020. Probiotics (direct-fed microbials) in moultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: a systematic review. Animals 10:1863.

Kalavathy, R., N. Abdullah, S. Jalaludin, and Y. W. Ho. 2003. Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br. Poult. Sci. 44:139–144.

Klandorf, H., S. B. Holt, J. A. McGowan, Y. Pinchasov, D. Deyette, and R. A. Peterson. 1995. Hyperglycemia and non-enzymatic glycation of serum and tissue proteins in chickens. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 110:215–220.

Knudsen, K. E. B. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci. 93:2380–2393.

Kuttappan, V. A., Y. S. Lee, G. F. Erf, J.-F. C. Meullenet, S. R. McKee, and C. M. Owens. 2012. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poult. Sci. 91:1240–1247.

Larsen, I. S., B. S.-Y. Choi, B. Föh, N. N. Kristensen, A. Ouellette, R. F. Haller, P. B. Olsen, D. Saulnier, C. Sina, B. A. H. Jensen, and A. Marette. 2023. Experimental diets dictate the metabolic benefits of probiotics in obesity. Gut Microbes 15:2192547.

Lazaro, R., M. Garcia, P. Medel, and G. Mateos. 2003. Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. Poult. Sci. 82:132–140.

Li, T., B. Liu, K. Chen, Y. Lou, Y. Jiang, and D. Zhang. 2020. Small molecule compounds promote the proliferation of chondrocytes and chondrogenic differentiation of stem cells in cartilage tissue engineering. Biomed. Pharmacother. 131:110652.

Liu, F., A. Kong, P. Fu, Q.-Q. Cao, K.-S. Tao, D.-Y. Liu, X.-B. Wang, Z.-X. Tong, M. U. Rehman, and S.-C. Huang. 2021. Lactobacillus rhamnosus JYLR-005 prevents thiram-induced tibial dyschondroplasia by enhancing bone-related growth performance in chickens. Probiotics Antimicrob. Proteins 13:19–31.

Liu, Y., C. D. Espinosa, J. J. Abelilla, G. A. Casas, L. V. Lagos, S. A. Lee, W. B. Kwon, J. K. Mathai, D. M. D. L. Navarro, N. W. Jaworski, and H. H. Stein. 2018. Non-antibiotic feed additives in diets for pigs: a review. Anim. Nutr. 4:113–125.

Lumeij, J. T. 2008. Chapter 28 - avian clinical biochemistry. Pages 839−872 in Clinical Biochemistry of Domestic Animals. J. J. Kaneko, J. W. Harvey and M. L. Bruss, eds. 6th Edition. Academic Press, San Diego.

Mehrabadi, M., and R. Jamshidi. 2019. Effect of antibiotic, probiotic and prebiotic in diets containing barley on performance, digestibility, intestinal morphology, blood parameters and immunological response in broilers. Iran. J. Appl. Anim. Sci. 9:497–507.

Mignon-Grasteau, S., S. Beauclercq, S. Urvoix, and E. Le Bihan-Duval. 2020. Interest in the serum color as an indirect criterion of selection of digestive efficiency in chickens. Poult. Sci. 99:702–707.

Nguyen, H. T., M. R. Bedford, and N. K. Morgan. 2021. Importance of considering non-starch polysaccharide content of poultry diets. World Poult. Sci. J. 77:619–637.

OECD, Food and Agriculture Organization of the United Nations. 2019. OECD-FAO Agricultural Outlook 2019-2028. OECD-FAO Agricultural Outlook. OECD.

Pérez, S. L., J.-P. Lallès, and J. P. Suescún. 2016. Effect of probiotic strain addition on digestive organ growth and nutrient digestibility in growing pigs. Rev. Fac. Nac. Agron. Medellín 69:7911–7918.

Piotrowska, A., K. Burlikowska, and R. Szymeczko. 2011. Changes in blood chemistry in broiler chickens during the fattening period. Folia Biol 59:183–187.

Piqué, N., M. Berlanga, and D. Miñana-Galbis. 2019. Health benefits of heat-killed (yyndallized) probiotics: an overview. Int. J. Mol. Sci. 20:2534.

Polovinski-Horvatović, M. 2021. A mini review of the effects of NSP and exogenous enzymes in broiler diets on digestibility and some intestinal functions. Serb. J. Agric. Sci. 70:116–122.

Proszkowiec-Weglarz, M., and R. Angel. 2013. Calcium and phosphorus metabolism in broilers: effect of homeostatic mechanism on calcium and phosphorus digestibility. J. Appl. Poult. Res. 22:609–627.

Rath, N. C., G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Factors regulating bone maturity and strength in poultry. Poult. Sci. 79:1024–1032.

Selim, S., N. S. Abdel-Megeid, H. K. Khalifa, K. G. Fakiha, K. A. Majrashi, and E. Hussein. 2022. Efficacy of various feed additives on performance, nutrient digestibility, bone quality, blood constituents, and phosphorus absorption and utilization of broiler chickens fed low phosphorus diet. Animals 12:1742.

Shastak, Y., and M. Rodehutscord. 2015. A review of the role of magnesium in poultry nutrition. World Poult. Sci. J. 71:125–138.

Sihvo, H.-K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623.

Sophocleous, A., A. Azfer, C. Huesa, E. Stylianou, G. R. Blanco, and S. H. Ralston. 2020. Probiotics prevent cartilage damage and progression of osteoarthritis in mice. Bone Reports 13:100666.

Swiatkiewicz, S., A. Arczewska-Wlosek, and D. Jozefiak. 2017. The nutrition of poultry as a factor affecting litter quality and foot pad dermatitis - an updated review. J. Anim. Physiol. Anim. Nutr. 101:e14–e20.

Tellez, G., J. D. Latorre, V. A. Kuttappan, M. H. Kogut, A. Wolfenden, X. Hernandez-Velasco, B. M. Hargis, W. G. Bottje, L. R. Bielke, and O. B. Faulkner. 2014. Utilization of rye as energy source affects bacterial translocation, intestinal viscosity, microbiota composition, and bone mineralization in broiler chickens. Front. Genet. 5.

Valchev, I., D. Kanakov, T. Hristov, L. Lazarov, R. Binev, N. Grozeva, and Y. Nikolov. 2014. Effects of experimental aflatoxicosis on renal function in broiler chickens. Bulg. J. Vet. Med. 17:314–324.

van Krimpen, M. M., M. Torki, and D. Schokker. 2017. Effects of rye inclusion in grower diets on immune competence-related parameters and performance in broilers. Poult. Sci. 96:3324–3337.

Vertiprakhov, V. G., A. A. Grozina, V. I. Fisinin, and I. A. Egorov. 2018. The correlation between the activities of digestive enzymes in the pancreas and blood serum in chicken. Open J. Anim. Sci. 8:215–222.

Vinderola, G., M. E. Sanders, and S. Salminen. 2022. The concept of postbiotics. Foods 11:1077.

Wastyk, H. C., D. Perelman, M. Topf, G. K. Fragiadakis, J. L. Robinson, J. L. Sonnenburg, C. D. Gardner, and E. D. Sonnenburg. 2023. Randomized controlled trial demonstrates response to a probiotic intervention for metabolic syndrome that may correspond to diet. Gut Microbes 15: 2178794.

Wu, Y., B. Wang, Z. Zeng, R. Liu, L. Tang, L. Gong, and W. Li. 2019. Effects of probiotics Lactobacillus plantarum 16 and Paenibacillus polymyxa 10 on intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. Poult. Sci. 98:5028–5039.

Yazhini, P., P. Visha, P. Selvaraj, P. Vasanthakumar, and V. Chandran. 2018. Dietary encapsulated probiotic effect on broiler serum biochemical parameters. Vet. World 11:1344–1348.

Zaefarian, F., M. R. Abdollahi, A. Cowieson, and V. Ravindran. 2019. Avian liver: the forgotten organ. Animals 9:63.

Zhu, C., L. Gong, K. Huang, F. Li, D. Tong, and H. Zhang. 2020. Effect of heat-inactivated compound probiotics on growth performance, plasma biochemical indices, and cecal microbiome in yellow-feathered broilers. Front. Microbiol. 11:585623.