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Lactobacilli-Based Pro and Postbiotic Efficacy Are Also Influenced by Other Factors Than Dietary Challenging Conditions

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ABSTRACT

The present study aimed to confirm the previously reported 'recovery' effect to a challenging diet (CD) of a *Lactobacilli*-based probiotic (Pro) and its derived postbiotic (Post) in broilers. Identical diet compositions were used, and observations were extended to a second CD diet. A completely randomised block design of 2 × 3 treatment groups with two CDs and three additive conditions (Control, Pro, Post) was used. One additional group received a standard diet (SD). The study involved 1600 one-day-old Ross 308 male broilers. All diets, fed from d1 to 35, were formulated to contain identical nutrients levels, with CDs formulated to be greater than SD in nonstarch polysaccharides using rye and barley (Rye CD) or dry distiller grains with soluble, sunflower and rapeseed meal (DDGS CD). Growth performance parameters, footpad lesions (FPL) score and plasma Ca, P and uric acid concentrations were measured. Compared to SD, birds fed Rye CD and DDGS CD had a higher 1–35 days feed conversion ratio (+3.4 and +4.1%, respectively), due to a higher feed intake for Rye CD (+2.9%) and a lower body weight for DDGS CD (−4.1%). An effect of additive was restricted to Rye CD where Post depressed BW at d28 and d35 (−3.7 and −2.4%, respectively). Compared to Rye CD, DDGS CD lowered plasma Ca/P at d21 (−9.0%) and d35 (−8.1%) and uric acid at d21 (−26%). Pro increased plasma Ca in Rye CD at d21 (+12%) and Post decreased plasma uric acid in DDGS CD at d35 (−25%). All other plasma parameters were not affected. The previously observed recovery effect of a commercial probiotic and postbiotic were not reproducible under highly similar growth conditions, which suggests that both may have specific physiological effects which are only expressed under specific circumstances.

1 | Introduction

Ingredients for poultry feed are becoming scarcer and more expensive (Singh and Kim 2021) encouraging the use of alternative ingredients. Inclusion of the latter can result in more challenging dietary conditions, and subsequently can have a negative impact on broiler health, growth performance and welfare. To favourable impact the growth and health in broilers, probiotics and their derivatives (postbiotics) are often used (Fathima et al. 2022; Jha et al. 2020). Both pro- and postbiotics can elicit beneficial effects associated with gut health and microbiota composition, fermentation and immunity (Fathima et al. 2022; Liu et al. 2018; Piqué et al. 2019). However, their effects on the performance of broilers, but also of pigs, appear to be inconsistent (Blajman et al. 2014; Fathima et al. 2022; Liu et al. 2018).

We recently reported a diet-dependent effect of pro- and postbiotics in broilers; supplementation of a commercial *Lactobacilli*-based probiotic and its derived postbiotic to a soy, wheat and maize (standard) or rye and barley (challenge) diet, improvement in BW was observed with the challenge, but not the standard diet (Jansseune et al. 2024). We also found diet-dependent and independent effects on blood plasma parameters (Jansseune et al. 2024). A dietary effect on the efficacy of probiotics has also been reported for other species, including mouse, pig and human as well as laying hens (Abd El-Hack et al. 2017; Larsen et al. 2023; Liu et al. 2018; Wastyk et al. 2023). In pigs, for instance, it was suggested that the response to probiotics depends on the type and quantity of dietary fibres (Liu et al. 2018). This role of fibre may be attributed to their fermentation by the gut microbiota and by probiotics, yielding a variety of biologically active end-products, such as short-chain fatty acids (SCFA) (Fathima et al. 2022; Singh and Kim 2021).

Fibres are present in most feed ingredients, but there is a large variability in terms of quantity and characteristics. For example, rye, barley, corn DDGS, rapeseed meal and sunflower meal (Knudsen 2014; Lannuzel et al. 2022; Polovinski-Horvatović 2021) have particularly high levels of nonstarch polysaccharides (NSP), which are known to impair digestibility, gut health, immunity and increase the risk of colonization by intestinal pathogens (Bindari and Gerber 2022; Immerseel et al. 2004; van Krimpen et al. 2017). As such, diets formulated partially with rye and barley as energy sources or DDGS, rapeseed meal and sunflower meal as protein sources can elicit challenging conditions for broiler growth and gut health, allowing measurement of a recovery effect of pro- and postbiotics supplements.

In the present study, we aimed to confirm the previously reported 'recovery' effect of a *Lactobacilli*-based probiotic (Pro) and its derived postbiotic (Post) in broilers under identical dietary challenging conditions (challenge diet [CD] containing rye and barley [Rye CD]) and to extend the observation to another dietary challenging condition where DDGS, rapeseed meal and sunflower, (DDGS CD) are used as ingredients. We hypothesized that dietary challenging conditions allow Pro and Post to 'recover' the loss in growth performance, compared to a standard corn, wheat and soybean meal-based diet (SD).

2 | Materials and Methods

2.1 | Ethics Statement

The protocol for the present broiler experiment 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.

2.2 | Birds Housing and Management

A large batch of day-old male Ross 308 broilers originating from 30-week-old laying hens was purchased from a commercial hatchery (Couvoir Goasduff, Plabennec, France). Based on individual weights, 1600 chicks were selected and distributed across 40 pens with 40 broilers each, so that all pens had a similar average chick body weight (BW) (~38.2 g) and distribution. Pens (1.90 × 1.25 × 0.8 m [L × W × H]) with wood shavings as floor covering were located along the wall of air entries on one side of a commercial, 1200 m² Colorado type building. Water and feed per pen were provided *ad libitum* through nipple drinkers ($n = 5–6$) and one 40 cm diameter Hung pan feeder (Josse, Montauban de Bretagne, France). The photoperiod was 24 h light until d4 and then 20 h from d5 to d42. Ambient temperature started at 32°C and, thereafter, gradually reduced in a linear fashion to 23°C at d22. Birds were daily inspected for lethargy, prostration and lameness and culled if found to be unhealthy.

2.3 | Experimental Treatments

A completely randomised block design of 2 × 3 treatment groups with two diets and three additives was used with six pens per treatment distributed among six blocks. One additional control standard diet (SD) treatment was represented in four pens distributed among four blocks. The three pelleted diets (SD, Rye and DDGS CDs) 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 (Tables 1 and 2), including apparent metabolizable energy, crude protein, essential amino acids and minerals. Pellet width was 2.5 mm for the starter and grower diets and 3.0 mm for the finisher diets. The SD was formulated to contain corn, wheat, and soybean meal with the control (Ctrl)-Rye CD containing also rye, barley and palm oil fat (except for starter phase), and the Ctrl-DDGS CD containing also DDGS, rapeseed meal and sunflower meal. The two CDs were either unsupplemented or supplemented with a commercial probiotic additive SORBIFLORE (referred to as Pro) and its derived postbiotic additive METALAC (STI biotechnologie, Maen Roch, France) (referred to as Post). 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 after fermentation and were dried as powder on carriers. Pro and Post biomasses were included in the diet at 50 and 500 g biomass/t as is, respectively, from d1 to d14, and at 40 and 400 g biomass/t as is, respectively, from d15 to d35.

2.4 | Data Collection

Individual BW and average feed intake per pen (FI) were recorded and feed conversion ratio (FCR) calculated at d14, d21, d28 and d35. Dead birds were collected daily and weighed to adjust pen FCR. At d35, footpad lesions (FPL) were scored in all remaining birds with grade 0 allocated for no lesion, grade 1 for mild lesions (altered lesion covers ≤ 25% of the footpad) and grade 2 for severe lesions (altered lesion covers > 25% of the footpad).

Blood sampling was performed by wing vein puncture on four randomly selected broilers/pen from six pens per treatment of CDs, at d21 and d35, with heparin as anticoagulant. Blood plasma was

TABLE 1 | Ingredient composition of the standard (SD), rye/barley (Rye) and dried distiller grains with soluble (DDGS) starter, grower and finisher diets for broilers.

	Starter (0–14 days)			Grower (14–28 days)			Finisher (28–35 days)		
Ingredient (% as is)	**SD**	**Rye**	**DDGS**	**SD**	**Rye**	**DDGS**	**SD**	**Rye**	**DDGS**
Corn	29.600	16.400	12.600	35.200	15.800	16.800	42.650	19.659	27.950
Wheat	30.034	27.130	34.955	29.950	27.977	36.650	30.000	26.100	35.100
Barley	—	10.000	—	—	10.000	—	—	10.000	—
Rye	—	5.000	—	—	10.000	—	—	15.000	—
Corn DDGS	—	—	15.200	—	—	13.800	—	—	12.000
Soybean meal	33.600	33.500	20.500	28.800	28.400	15.500	21.700	21.300	10.800
Rapeseed meal	—	—	4.000	—	—	5.100	—	—	3.500
Sunflower meal	—	—	3.500	—	—	3.500	—	—	3.000
Limestone	1.580	1.568	1.490	1.090	1.100	0.988	0.806	0.820	0.734
Mono calcium phosphate dihydrate	1.380	1.330	1.340	0.910	0.840	0.870	0.790	0.700	0.750
Soy oil	2.450	3.710	4.000	2.740	4.000	4.000	2.630	4.000	4.000
Palm oil	—	—	0.580	—	0.550	1.000	—	0.980	0.340
Sodium chloride 99%	0.280	0.280	0.220	0.240	0.240	0.200	0.244	0.240	0.200
Sodium bicarbonate	0.100	0.100	0.100	0.120	0.120	0.120	0.120	0.120	0.120
DL-methionine 99%	0.242	0.251	0.235	0.217	0.225	0.206	0.199	0.209	0.19
L-lysine HCl 98%	0.153	0.147	0.388	0.162	0.159	0.392	0.249	0.242	0.444
L-threonine 98%	0.081	0.084	0.130	0.071	0.079	0.118	0.097	0.106	0.139
L-tryptophan 98%	—	—	0.028	—	—	0.022	—	—	0.020
L-isoleucine 90%	—	—	0.049	—	0.010	0.053	—	0.011	0.046
L-arginine 98%	—	—	0.185	—	—	0.181	0.015	0.013	0.167
Premix[a]	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500

[a]Supplied per kg: 2,000,000 IU retinyl acetate, 500,000 IU cholecalciferol, 10 g DL-a-tocopherol, 460 mg menadione, 400 mg thiamine, 1500 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 $FeSO_4 \cdot H_2O$, 9.6 g $CuSO_4 \cdot 5H_2O$, 28 g MnO, 33 g $ZnSO_4 \cdot H_2O$, 360 mg KJ, 112 mg Na_2SeO_3.

obtained before storage at −20°C. Total Ca, P and uric acid were quantified by Arsenazo III, phosphomolybate and uricase-peroxidase methods, respectively, with a ProVet analyser according to the manufacturer's protocol (Kitvia, Labarthe-Inard, France).

2.5 | Statistical Analyses

All analyses were performed with R version 4.0.3 (R Core Team 2023). Probability values < 0.05 were considered as being significant and a trend defined as $0.05 \le p < 0.10$. First, values deviating by more than three standard deviations from the mean were considered as outliers and excluded from the data set. Excluded values were restricted to Ca (one value) and uric acid (two values) at d21 with a maximum of one excluded value per treatment.

Treatment effects (CDs and additives) on means were analysed using the general linear model procedure and type 2 analysis of variance (ANOVA), whereas their effect on FPL score was analysed by ordinal logistic regression. Pens were the statistical units and, when applicable, individual birds were the observational units. The statistical models included fixed effects of CDs and additives, their interaction and a random block factor. For blood parameters, BW was included as an additional fixed effect. For the linear models, variance homogeneity was assessed with Levene's test and, in case of heteroscedasticity, a White-Huber correction was applied. Additionally, if residuals were not normally distributed, a Box-Cox transformation was applied with normality of residuals confirmed by Shapiro test. If residuals normality was not reached, a permutational analysis of variance was applied (vegan package v2.6-4) with 5000 permutations and Euclidian distance as proposed by Anderson (2017). For all statistical models, when an interaction effect was significant, intra-diet contrast (Pro vs. Ctrl and Post vs. Ctrl) and Ctrl CD comparison were performed. When a main additive effect was significant, interdiet contrast analyses were performed to assess the overall effect of Pro (Ctrl vs. Pro) and Post (Ctrl vs. Post). To assess the effect of the CDs compared to the SD, the above-described procedure was applied on contrasts for Ctrl-Rye CD versus SD and Ctrl-DDGS CD versus SD with exclusion of the additive effect parameter.

3 | Results

Broiler growth performance parameters were affected by dietary treatments (Table 3). Compared to the SD, the Ctrl Rye and DDGS CDs fed birds had a greater d1–35 FCR (+3.4 and +4.1%,

TABLE 2 | Calculated nutrient composition including energy content of the standard (SD), rye/wheat (Rye) and dried distiller grains with soluble (DDGS) starter, grower and finisher diets for broilers.

	Starter (d0–14)			Grower (d14–28)			Finisher (d28–35)		
Composition (% as is)	**SD**	**Rye**	**DDGS**	**SD**	**Rye**	**DDGS**	**SD**	**Rye**	**DDGS**
Dry matter	88.1	88.4	88.7	87.9	88.3	88.5	87.7	88.2	88.2
Crude protein	21.9	21.9	21.9	20.0	20.0	20.0	17.3	17.3	17.3
Crude fat	4.12	5.16	6.91	4.51	6.00	7.31	4.53	6.51	6.74
Starch	38.7	36.6	31.3	42.2	39.5	35.0	47.0	43.5	41.1
Ash	5.89	5.91	5.90	4.75	4.79	4.78	4.04	4.08	4.06
Fibre[a]	12.9	14.0	14.7	12.4	13.7	14.2	11.6	13.2	13.1
Total NSP[a,b]	11.5	12.5	12.9	11.1	12.2	12.3	10.3	11.6	11.4
Soluble NSP[a]	2.64	3.05	2.46	2.45	2.96	2.28	2.15	2.76	2.01
Cellulose	2.77	2.93	4.42	2.75	2.86	4.44	2.70	2.77	4.08
Dig. Methionine	0.55	0.55	0.55	0.50	0.50	0.50	0.45	0.45	0.45
Dig. Methionine + cystine	0.90	0.90	0.90	0.83	0.82	0.83	0.74	0.74	0.74
Dig. Lysine	1.16	1.16	1.16	1.05	1.05	1.05	0.95	0.95	0.95
Dig. Threonine	0.78	0.78	0.78	0.70	0.70	0.70	0.64	0.64	0.64
Dig. Valine	0.90	0.90	0.89	0.82	0.82	0.82	0.71	0.71	0.71
Dig. Arginine	1.33	1.33	1.32	1.19	1.19	1.19	1.00	1.00	1.00
Dig. Tryptophan	0.24	0.25	0.24	0.22	0.22	0.21	0.18	0.19	0.18
Dig. Isoleucine	0.82	0.81	0.82	0.74	0.74	0.74	0.63	0.63	0.63
Calcium	0.94	0.94	0.94	0.66	0.66	0.66	0.51	0.51	0.51
Available phosphorous	0.46	0.46	0.46	0.34	0.34	0.34	0.30	0.30	0.30
Chlorine total	0.25	0.25	0.28	0.23	0.23	0.27	0.24	0.24	0.27
Sodium total	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14
Apparent metabolizable energy (MJ/kg as is)	12.03	12.03	12.03	12.60	12.60	12.60	12.87	12.87	12.87

[a]Values calculated from Knudsen (2014) and Pedersen et al. (2014) and restricted to cereals, DDGS and meals.
[b]Nonstarch polysaccharides.

respectively). In Ctrl Rye, this FCR increase occurred concomitantly with an increase in daily FI (+2.9%), but no change in BW was observed. However, for Ctrl DDGS CD, BW was lower (−4.1%), but daily FI was not affected. Moreover, compared to Ctrl Rye CD, birds fed the Ctrl DDGS CD had a lower BW at d35 (−3.0%) as well as a higher FCR and lower FI for period between d29 and 35 (+3.2 and −3.2%, respectively). Subsequently, the d29–35 BW was 6.2% lower in Ctrl DDGS CD compared to Ctrl Rye CD.

An effect of the additive was restricted to Rye CD in which Post depressed BW at d28 and d35 (−3.7% and −2.4%, respectively). Mortality, as shown by the mean number of birds removed per pen, was not affected by dietary treatments and averaged 2.1 birds/pen at d35, a value equivalent to those found at the same facilities in other experiments (data not provided). Mean FPL score was affected by CDs and additives. The DDGS CD reduced FPL compared to SD and Rye CD (−61% and −59%, respectively). Moreover, Pro increased the mean FPL score (+35%, $p < 0.001$), whereas postdecreased it (−13%, $p = 0.013$).

Blood plasma parameters were affected by dietary treatments (Table 4). Compared to Rye CD, birds fed DDGS CD had a lower Ca/P at both d21 (−9.0%; $p < 0.001$) and d35 (−8.1%; $p < 0.001$). Compared to Rye CD, the DDGS CD group had also a lower plasma uric acid at d21 (−26%; $p < 0.001$). No fixed effect of additive treatment was observed on all plasma parameters but Pro increased plasma Ca in Rye CD at d21 (+12%; $p = 0.040$) and Postdecreased plasma uric acid in DDGS CD at d35 (−25%; $p = 0.046$). All other parameters were not affected.

4 | Discussion

Here, we aimed to confirm the previously reported 'recovery' effect to a challenging diet of a *Lactobacilli*-based probiotic and its derived postbiotic in broilers. The present study was conducted in the same production facilities, under the same care and under identical dietary challenging conditions (Rye CD). In addition, the objective was to extend the observation to another dietary challenging condition where DDGS, rapeseed meal and sunflower (DDGS CD) are used as ingredients. We hypothesized that dietary challenging conditions allow the previously observed 'recovery' effect of Pro and Post when performance is reduced. Unexpectedly, no beneficial effect of Pro and Post were observed, although a clear decrease in performance of Rye and DDGS CDs was

TABLE 3 | Mean individual body weight and footpad lesion score, pen daily feed intake, feed conversion ratio and birds removed of male Ross 308 broilers fed a standard (SD), rye/barley (Rye) or dried distiller grains with soluble (DDGS) control (Ctrl) diet supplemented with *Lactobacilli*-based probiotic (Pro) and postbiotic (Post) from Day 1–35.

	Diet[a]								*p*-value[b]		
		Rye			DDGS						
Parameter	**SD**	**Ctrl**	**Pro**	**Post**	**Ctrl**	**Pro**	**Post**	**Pooled SEM**	**Diet**	**Additive**	**Diet × additive**
Body weight (g)											
d14	343	339	337	341	328^{+}	321	334	8	< 0.001	0.007	0.320
d21	796	786	772	770	777	767	787	18	0.816	0.168	0.097
d28	1379	1367	1352	1317^{ℙℙ}	1353	1329	1357	32	0.930	0.095	0.012
d35	2070	2048	2047	1999^{ℙ}	1986$^{+,*}$	1991	2016	45	0.020	0.713	0.036
Daily feed intake (g as is)											
d1–11	27.5	27.8	27.5	28.3	27.1	27.0	27.6	1.95	0.154	0.437	0.985
d12–21	86.1	87.4	85.6	86.1	87.2	87.6	86.8	3.6	0.366	0.718	0.710
d22–28	122.3	127.4	126.6	121.1	124.8	126.8	125.7	4.9	0.652	0.199	0.170
d29–35	175.8	181.0	181.6	179.7	175.1	174.6	175.3	5.5	0.001	0.948	0.826
d1–35	88.7	91.3	90.8	89.7	88.5	89.9	89.6	2.4	0.128	0.773	0.382
Feed conversion ratio (g/g)											
d1–11	1.045	1.073	1.073	1.085	1.087^{+}	1.097	1.082	0.029	0.170	0.894	0.471
d12–21	1.333	1.373	1.378	1.407	1.358^{+}	1.375	1.348	0.048	0.124	0.846	0.301
d22–28	1.470	1.538^{+}	1.537	1.563	1.530^{+}	1.580	1.548	0.062	0.673	0.552	0.314
d29–35	1.815	1.865	1.843	1.847	1.930^{+}	1.890	1.885	0.077	0.046	0.521	0.907
d1–35	1.480	1.530^{+}	1.525	1.542	1.540^{++}	1.553	1.530	0.021	0.173	0.861	0.040
Birds removed per pen											
d35	2.8	2.2	1.8	1.5	3.0	1.7	2.0	1.7	0.411	0.252	0.680
Footpad lesion score											
d35	1.04	0.916	1.243	0.857	0.406^{+}	0.545	0.297	0.137	< 0.001	< 0.001	0.1831

Note: $^{+, ++}$ indicates the mean of Ctrl are different from the Ctrl of the SD at $p < 0.05$ and $p < 0.001$, respectively. $^{ℙ, ℙℙ}$ indicates the means for Post and Pro are different from their respective intradiet Ctrl at $p < 0.05$ and $p < 0.001$, respectively. Conducted in case of a significant interaction.

[a]Body weight and footpad lesion score values are the mean of individual measures while values for daily feed intake, feed conversion ratio and number of birds removed are the mean of six pen replicates per treatment but four for SD.

[b]ANOVA was performed on the Rye and DDGS-diets × additive (Ctrl, Pro and Post). The SD was not included in this analysis.

*Mean of Ctrl Rye are different from the Ctrl DDGS at $p = 0.005$.

TABLE 4 | Mean calcium (Ca), phosphorus (P) and uric acid concentration in plasma of 21- and 35-day-old male Ross 308 broilers fed rye/wheat (Rye) or dried distiller grains with soluble (DDGS) control (Ctrl) diet supplemented with *Lactobacilli*-based probiotic (Pro) and postbiotic (Post) from Day 1–35.

	Diet[a]							*p*-value[b]		
	Rye			DDGS						
Parameter	Ctrl	Pro	Post	Ctrl	Pro	Post	Pooled SEM	Diet	Additive	Diet × additive
d21										
Uric acid (μM)	394	409	320	283	285	290	72	< 0.001	0.270	0.127
Ca (mM)	2.98	3.34[ℙ]	2.79	3.04	2.91	3.06	0.35	0.691	0.282	0.018
P (mM)	2.99	3.28	2.63	3.31	3.07	3.16	0.43	0.086	0.141	0.048
Ca/P	1.01	1.04	1.07	0.93	0.96	0.95	0.06	< 0.001	0.120	0.679
d35										
Uric acid (μM)	351	329	412	336	304	252[ℙ]	74	0.004	0.665	0.014
Ca (mM)	3.94	3.62	3.96	3.66	3.75	3.37	0.54	0.121	0.548	0.344
P (mM)	3.82	3.33	3.74	3.87	3.90	3.47	0.62	0.595	0.367	0.263
Ca/P	1.04	1.09	1.07	0.96	0.98	1.00	0.06	< 0.001	0.096	0.382

[a]Values are means of four animals/pen and six broilers per pen, excluding outliers.
[b]*p*-values were body weight corrected in the ANOVA model.
[ℙ]Means for Post and Pro are different from their respective intra-diet Ctrl at $p < 0.05$. Conducted in case of a significant interaction.

observed compared to the SD. The results of the present study do, therefore, not support the hypothesis and are contradictory to our previously observed effects (Jansseune et al. 2024).

In that previous study by Jansseune et al. (2024), Pro and Post improved broiler growth in a rye-based challenge diet with a formulation almost identical (except for the finisher diet containing less wheat and more corn) to the Rye diet. The DDGS CD was formulated to also yield a dietary challenge as a result of a higher cellulose content than SD, and Rye CD was formulated to be challenging due to a higher soluble NSPs content. No Pro and Post effect on growth performance parameters was observed for DDGS CD, but as this was the same for Rye CD, it cannot be concluded that the absence of a 'recovery' effect of Pro and Post for DDGS CD was due to the specificity of this diet (cellulose-rich diet for DDGS CD vs. soluble NSPs-rich diet for Rye CD). The effects of Pro on plasma parameters are also inconsistent with our previous study (Jansseune et al. 2024), whereas in both experiments, Post had no effects on plasma Ca, P and uric acid concentration. In the previous study, Pro increased plasma Ca, P and uric acid at both d21 and d35 (Jansseune et al. 2024). In the current study, Pro only increased plasma Ca at d21. The lack of reproducible biological effect is often pointed out for pro- and postbiotics (Blajman et al. 2014; Fathima et al. 2022; Liu et al. 2018), but comparisons between studies are complex because of variable conditions (e.g., sanitary status, country, diet composition, birds, housing, etc.). Compared to the current study, in the previous one (Jansseune et al. 2024), 1 year earlier, birds were the same breed, housed in the same barn with identical housing conditions and fed diets with identical ingredients. The studies differed only in terms of the level of certain ingredients (mainly wheat and corn in the finisher diet), batches of ingredients, batches of Pro and Post, broiler breeders, sanitary pressure, and weather conditions. The results here indicate that the understanding of Pro and Post effects requires more in-depth knowledge of their precise mode of action.

It must be pointed out that the broilers in the study here showed a better growth performance than in our previous study (Jansseune et al. 2024), despite that for both studies the diets were formulated with identical levels of nutrients and used highly similar ingredient compositions. The growth performance of birds was in line with those of male Ross 308 reported in the literature (Bedford et al. 2018; Martínez and Valdivié 2021; Qaisrani et al. 2020). The chicks in the current study had a lower initial BW than in the previous study (38.2 vs. 43.0 g; −11%; Jansseune et al. 2024). This lower initial BW would have negatively affected broiler BW at slaughter age. However, compared to the previous study, the BW of broilers fed the SD here was lower up to d21 (−52 g; −6.1%), similar at d28 (−21 g; −1.5%) and greater at d35 (+67 g; +3.3%). A more pronounced pattern of difference between experiments was observed in birds fed Rye CD which had a lower BW in the current experiment up to d21 (−68 g; −8.0%), a similar BW at d28 (−14 g; −1.0%) and a greater BW at d35 (+112 g; +5.8%). Moreover, compared to the results presented by Jansseune et al. (2024), the d1–35 FCR was lower in the current study by −0.061 (−4.0%) and −0.089 (−5.5%) for the SD and Rye CD, respectively. Moreover, Rye CD had a less adverse effect on growth performances than in the previous experiment, in which it reduced broiler BW at d35 (Jansseune et al. 2024). Of note, in the latter study is that birds reared in the commercial flock showed around d20 excreta indicators associated with poor gut health, indicating that a health challenge may have occurred in the barn. Altogether, these elements on growth performance and potential health challenge suggest that the birds in the current study had a better health status than the ones in the previous experiment. This would imply that the health status of the birds is likely to be important for the effects of Pro and Post, with Rye creating a favourable context to show Pro and Post effects.

The two CDs were formulated to contain the same level of nutrients as the SD, but depressed broiler growth performance as shown by higher FCR. This difference in FCR was due to a higher FI in birds fed the Rye CD and a lower BW in birds receiving the DDGS CD. Our results suggest that birds fed Rye CD could have maintained their growth by increasing their FI. Such increase of FI relative to BW was also observed in birds fed DDGS CD, as shown by a +4.6% of the d29–35 FCR, but this was not enough to maintain broiler growth. Of note, birds fed DDGS CD had comparable growth performances compared to birds fed Rye CD up to d28 but showed a reduced performance during the period between d29–35 as shown by lower BW and higher FCR. This effect of DDGS CD in the finisher phase may partly originate from a lack of available lysine since DDGS is highly variable in this essential nutrient (Pahm et al. 2008) and since the DDGS ingredient was calculated to contribute to 6.6%, 7.3% and 10.0% of the digestible lysine supply in the starter, grower and finisher diets, respectively.

The CDs-induced increase in FCR compared to the SD likely originated from the inclusion of rye and barley and from the inclusion of DDGS, sunflower meal and rapeseed meal. Rye, barley, DDGS, sunflower meal and rapeseed meal contain higher concentrations of NSPs and a high proportion of soluble NSPs compared to wheat, corn and soybean meal (Knudsen 2014; Lannuzel et al. 2022). These polysaccharides and particularly soluble NSPs 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. Viscosity and water holding capacity can affect feed passage rate, but this effect depends on the physical and chemical properties and quantity of NSPs (Tejeda and Kim 2021). Some NSPs can also cause lipid malabsorption because of their bile acids, fat and cholesterol binding capacity (Tejeda and Kim 2021).

Interestingly, DDGS CD reduced FPL score when compared to the SD and Rye CD, while Pro increased FPL score and Post decreased it. The absence of an effect of Rye CD on FPL score, when compared to SD, is in accordance with the previous observation of Jansseune et al. (2024). Moreover, in the latter study, Pro and Post reduced mean FPL score in both a standard and a rye-based challenge diet, but with a more pronounced effect of Pro in the standard diet and Post in the rye-based challenge diet. This suggests that Post but not Pro could have a consistent effect on FPL reduction. Footpad lesions are promoted by high litter and excreta humidity and an increase of undigested protein excretion (Swiatkiewicz et al. 2017), which all are promoted by poor gut health (Hermans et al. 2006). Some of the latter parameters could have been improved by DDGS CD and Post but degraded by Pro.

The current results show that diets containing rye and barley or DDGS, rapeseed meal and sunflower meal can constitute dietary challenging conditions for broilers. Indeed, since the diets were formulated according to commercial standards, dietary nutrients were most likely sufficient and did not limit growth performance. The previously observed effects of a commercial probiotic and postbiotic under highly similar growth conditions was not reproducible, which suggests that both may have specific physiological effects which are only expressed under specific circumstances. This implies that a clear understanding of their mode of action is key for an optimal use of these types of additives.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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