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Feeding graded levels of dried Sea buckthorn (*Hippophaes rhamnoides*) berries to broiler chickens

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Abstract

The aim of this study was to assess the effects of graded levels (0, 3, 6, 9 and 12 g/kg) of dry Sea buckthorn (SB) berries on growth performance, gastrointestinal tract (GIT) development, jejunal histomorphology, bird antioxidant status and caecal short-chain fatty acid concentration when fed to female Ross 308 broiler chickens. In addition, expression of cytokine biomarker genes in the jejunum was evaluated. The five experimental diets were fed from 7 to 21 days age to 8 pens (two birds in each) following randomisation. Feeding SB did not influence bird growth performance ($p > .05$). There was a linear decrease in butyric, acetic and valeric acid concentrations in caecal digesta ($p < .05$) and a decrease ($p < .05$) in crypt depth. The expression of IFNG and CD40LG responded quadratically ($p < .05$), peaking at 6–9 g/kg dietary inclusion of SB, respectively. Other studied variables were not affected by dietary SB inclusion ($p > .05$). Feeding dry SB berries up to 12 g/kg of diet did not improve the zootechnical variables of healthy commercial-strain broilers in this study.

KEYWORDS
broiler chicken, gene expression, gut health, Sea buckthorn

1 | INTRODUCTION

The use of medicinal plants, such as herbs, spices and their extracts in poultry feed, is popular for their beneficial applications relating to immunomodulation and their natural antioxidant activity (Pirgozliev et al., 2021; Whiting et al., 2022; Yeung et al., 2019). Sea buckthorn (*Hippophaes rhamnoides*; SB), is a thorny, dioecious plant which usually grows in dry areas (Biswas et al., 2010). The berries of SB contain various active components, including vitamins, phenols, terpenes and tannins that have been used for medicinal and nutritional purposes in Europe and Asia for many centuries (Biswas et al., 2010).

SB has been used as a poultry feed supplement (Biswas et al., 2010; Pirgozliev et al., 2022), but the growth response of birds is variable and inconsistent between experiments. In some studies, feeding SB flavones improved broiler performance (Ma et al., 2015); in other studies, it reduced feed intake (FI; Zhao et al., 2012), or even lowered overall bird performance (Ben-Mahmoud et al., 2014). Research by Tkacz et al. (2019) and Tudor et al. (2019) showed that SB berries are rich in antioxidants and other bioactive ingredients, although their impact on the antioxidant status of poultry needs further investigation (Hsu et al., 2009; Solcan et al., 2013). There is also limited information on the impact of SB on gastrointestinal tract (GIT) development and caecal fermentation when fed to broiler chickens (Panaite et al., 2022; Umirbekova et al., 2019). Increasing evidence suggests that through interactions with the immune system, flavones of SB can modulate immune responses (Diandong et al., 2016; Mishra

et al., 2008). Since antibiotics should not be prescribed to promote animal growth, or substitute for poor husbandry/ hygiene, dietary immunomodulation is a key tool contributing to the enhancement of productivity and supporting gut health of farm animals (Kumar et al., 2011; Munyaka et al., 2012).

The mode of action and efficacy of dietary SB in relation to poultry gut health and production performance appears to be poorly understood and inconsistent. Thus, the primary objectives of this study were to compare broiler growth performance, including FI, weight gain (WG) and feed conversion ratio (FCR) and antioxidant status when feeding graded levels of a well-characterised sample of dried SB berries. GIT development, jejunal histomorphology and short-chain fatty acid (SCFA) concentrations in caecal digesta were also evaluated. In addition, the experiment examined the effect of SB on local expression of biomarker genes in the jejunum that may indicate modulation of the immune response and intestinal health of growing commercial-strain broiler chickens.

2 | MATERIALS AND METHODS

2.1 | Diets

Five wheat-maize-soy-based diets were offered to the birds during the experiment from 7 to 21 days age. The basal diet was formulated to meet breeder's recommendations (Aviagen Ltd.; Table 1). The basal diet was then split into five batches, and one of the batches was used as a control diet (C). The remaining four diets were the C supplemented with dry and milled SB berries at 3, 6, 9 or 12 g/kg at the expense of the basal diet. The SB berries were collected from the Troyan region of the Balkan Mountains, Bulgaria. Whole berries were air-dried and then preserved frozen (−20°C) for 3 months before being used in the experiment.

TABLE 1 Ingredient composition (g/kg 'as fed') of the basal diet.

Ingredient	Composition g/kg (8–21 days)
Maize	381.90
Wheat	200.05
Rapeseed ext '00'	50.00
Soya hipro	290.04
Soya oil	35.00
Lysine	3.00
Methionine	3.00
L Threonine	1.00
Monocal phosphate	15.00
Limestone	12.50
Salt	2.00
Sodium bicarbonate	2.50
Vitamin/mineral premix[a]	4.00
Phytase (Quantum Blue 5 g)	0.01
	1000
Calculated composition	
Metabolisable energy (MJ/kg)	12.69
Crude protein (g/kg)	211.1
Lysine (g/kg)	13.9
Methionine + cysteine (g/kg)	9.6
Calcium (g/kg)	9.7
Available phosphorus (g/kg)	4.8
Sodium (g/kg)	1.7

[a]Provided per kg feed: 2160 μg retinol, 75 μg cholecalciferol; 25 mg α-tocopherol, 1.5 mg menadione, 5 mg riboflavin, 8 mg pantothenic acid, 10 μg cyanocobalamin, 1.5 mg pyridoxine, 1.5 mg thiamine, 0.5 mg folic acid, 30 mg niacin, 60 μg biotin, 0.8 mg I, 10 mg Cu, 80 mg Fe, 0.3 mg Se, 80 mg Mn and 80 mg Zn (Target Feeds Ltd., Whitchurch, UK).

2.2 | Animals and experimental design

The study procedures were approved by Harper Adams University Reserach Ethics ommittee and reportted here in accordance with the ARRIVE 2.0 quidelines (Percie du Sert et al., 2020). Ninety-day-old female Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason Ltd.). On arrival, the chicks were housed in a concrete-based common floor pen measuring 140 cm × 150 cm, bedded with wood shavings, and fed a wheat-based proprietary starter mash until 7 days of age. Experimental diets were offered at 7 days of age, when 80 birds, excluding ill and malformed, were individually weighed and distributed into 40 cardboard bedded pens (0.4 m × 0.4 m; two birds per pen). Each diet was offered ad libitum in meal form to birds in eight pens following randomisation (with spatial blocking) using a random number generator. Rearing conditions met breeders' recommendations (Aviagen Ltd.). The experiment continued for 14 days, from 7 to 21 days of age. FI, WG and FCR of each pen (unit of replication) were determined for the experimental period. Birds were monitored regularly to ensure health and welfare. Sample size was determined a priori to detect a 5% difference in WG based on the method of Berndtson (1991).

2.3 | Sample collection

Bird FI, WG and FCR were determined on a pen basis at 7 and 21 days of age. At the end of the study, one bird per pen, selected at random, was electrically stunned and blood from the jugular vein was collected directly into heparin-coated tubes (BD Vacutainer®). The GIT organs, including the proventriculus and gizzard (PG), duodenum, pancreas, jejunum, ileum, ceca and liver, were weighed. Approximately 3 cm of the middle part of the jejunum, between the point of bile duct entry and Meckel's diverticulum, of each bird was fixed in 10% neutral buffered formalin for 24 h, before further processing and analysis of morphometry. Samples from the same part of the jejunum of each bird were collected and stored in RNA*later*® (Sigma-Aldrich) at −80°C before RNA extraction. Individual bird liver was weighed, freeze-dried, milled and stored at −80°C before antioxidant analysis. A pooled digesta sample from both ceca was collected from one bird in each pen, freeze-dried and stored at −80°C for SCFA analysis.

2.4 | Analysis of dietary nutrients and antioxidants

Dry matter (DM) and proximate analysis (crude protein, oil as ether extract and gross energy [GE]), in feed and SB samples were determined as previously described (Whiting et al., 2022; Yang et al., 2020). Non-starch polysaccharides (NSP) content in the basal diet and SB samples were determined following the methods of Englyst et al. (1994). Minerals in the basal diet and SB samples were determined following the method described by Tanner et al. (2002).

Determination of carotenoids, vitamin E and coenzyme Q10 in feed and liver samples was analysed as per Karadas et al. (2014).

The glutathione peroxidase (GSH-Px) concentration in blood plasma was measured following manufacturer recommendations (Randox Laboratories Ltd.).

2.5 | GIT development, jejunal morphometry and SCFAs in caecal digesta

Following humane killing, morphometric measurements, empty weight of GIT segments and liver weight of birds were obtained (Abdulla et al., 2016; Woods et al., 2021). Caecal digesta SCFA concentrations were analysed as described in Pirgozliev et al. (2021).

2.6 | Total RNA extraction, reverse transcription and quantitative real-time PCR

Extraction of RNA from macro-dissected jejunal tissue, subsequent reverse transcription and quantitative real time PCR were performed as per Pirgozliev, Mansbridge, Rose, Lillehoj, et al. (2019) with the following modifications: homogenisation used 600 µL QIAzol lysis reagent; lysates were centrifuged for 5 min at room temperature to pellet debris (no chloroform or pegGold PhaseTrap steps); RNA integrity (RIN) was >9 for all samples; 500 ng of RNA were reverse transcribed; reactions were pipetted robotically using a CAS-1200™ (Corbett Life Science); amplification parameters were: 95°C for 5 min followed by 40 cycles of 95°C for 5 s, 60°C for 1 s in a Rotor-Gene Q (Qiagen); genes of interest included cytokine/immune-related (CD40LG, IFNG, IL2, IL4, IL6, IL10 and IL18), mucin (MUC2) and tight junction related (CLDN4 and OCLN); additional assays (Table 4) were designed by qStandard (www.qstandard.co.uk) and were tested for specificity by electrophoresis, efficiency >95%, sensitivity to 10 copies/rxn, and linearity over 7 log by qPCR; three reference genes were identified (ACTB, B2M and PPIA).

TABLE 2 Determined chemical composition of basal diet and dried Sea buckthorn (SB) berries.

Determined values	Basal diet	SB
Dry matter (g/kg)	884	903
Gross energy (MJ/kg)	16.89	23.62
Crude fat (g/kg)	62	233
Crude protein (g/kg)	214	180
Coenzyme Q_{10} (µg/g)	1.3	3.1
Vitamin E (µg/g)	34.4	45.0
Total carotenoids (µg/g)	2.6	346.8
Ca (g/kg)	11.90	0.73
P (g/kg)	8.07	2.18

2.7 | Statistical analysis

Data were analysed using Genstat (18th Edition) statistical software (IACR Rothamsted). Comparisons among studied variables were performed by one-way ANOVA (with spatial blocks) incorporating orthogonal polynomial contrasts for linear (L) and quadratic (Q) responses to graded levels of SB in diets. In all instances, differences were reported as significant at $p < .05$. All data were checked for homogeneity of variances and normality before ANOVA.

3 | RESULTS

No mortalities occurred during the study. There were no significant differences in live weight at 7 days old (156.4 ± 11.09 g) across the treatment groups, before study commencement.

The analysed chemical composition of SB and the basal diet are presented in Tables 2 and 3. Feeding SB did not influence ($p > .05$) any of the growth performance variables (Table 3). There were no ($p > .05$) deviations from linear and quadratic relationships for any studied variable. Feeding SB did not affect ($p > .05$) the relative weight of GIT organs (Table 5). There was a linear increase in crypt depth (CD) ($p < .05$) with SB inclusion level and overall linear decrease in villus height/crypt depth ratio (VH:CD) (Table 6). Feeding SB did not change the hepatic antioxidants or the concentration of blood plasma GSH-Px ($p > .05$) (Table 7). Increasing the dietary levels of SB linearly reduced ($p < .05$) the concentration of caecal acetic acid (AA), butyric acid (BA) and valeric acid (VA), but did not affect propionic acid (PA) ($p > .05$) (Table 8).

Genes for IL2, IL4, IL6, IL10 and CLDN4 either failed to amplify or were below the detection limit and are not included in the results. One sample failed the QC and was excluded from all analyses. CD40LG gene expression (copies per reaction) responded quadratically ($p = .033$), peaking at 9 g/kg SB inclusion (Table 9). Similarly, IFNG gene expression also responded quadratically ($p = .034$), peaking at 6 g/kg. There were no effects of SB dietary inclusion on cytokine gene (IL18), mucin (MUC2) or tight junction (OCLN) expression (Table 8).

TABLE 3 Carbohydrates in different sugar fractions of the Sea Buckthorn (SB) berries and the basal diet (BD) (g/100 g)[a]

Carbohydrates	rha	fuc	ara	xyl	man	gal	glu	GlcA	GalA	g/100 g
Soluble NSP (SB)	0.10	0.00	0.60	0.30	0.10	0.35	0.40	0.20	1.80	3.85
Insoluble NSP (SB)	0.10	0.00	1.00	4.40	0.40	0.35	4.70	0.00	0.30	11.25
Total NSP (SB)	0.20	0.00	1.60	4.70	0.50	0.70	5.10	0.20	2.10	15.10
Soluble NSP (BD)	0.10	0.10	0.80	0.30	0.10	0.80	0.50	0.10	0.44	3.24
Insoluble NSP (BD)	0.00	0.00	1.10	1.30	0.30	0.70	2.20	0.00	0.34	5.94
Total NSP (BD)	0.10	0.10	1.90	1.70	0.40	1.50	2.80	0.10	0.78	9.38

Abbreviations: ara, arabinose; fuc, fucose; gal, galactose, GalA, galacturonic acid; GlcA, glucuronic acid; glu, glucose; man, mannose; NSP, non-starch polysaccharides; rha, rhamnose; xyl, xylose.
[a]All data are the results of a chemical analysis conducted in duplicate.

TABLE 4 RT-qPCR assays[a] for quantification of gene expression in *Gallus gallus* jejunal tissue.

Gene symbol	Gene	Accession number	Primer sequences (5′-3′)	Product length, bp	Location
MUC2	Mucin 2	NM_001318434	S—AGGAATGGGCTGCAAGAGAC	77	S—Exon 19
			A—GTGACATCAGGGCACACAGA		A—Exon 19
OCLN	Occludin	NM_205128	S—GTGGAGGAGTGGGTGAAGAAC	78	S—Exons 3 and 4
			A—CTTCTCCGAGTAGGCAAGCGT		A—Exon 4

Abbreviations: A, anti-sense primer; S, sense primer.
[a]Primer sequences are provided in the interest of transparency but remain the intellectual property of qStandard (www.qstandard.co.uk).

TABLE 5 Effect of dietary Sea Buckthorn (SB) inclusion level (g/kg) on broiler chicken growth performance from 7 to 21 days age.

Treatment	BW (g/b)	FI (g/b/d)	WG (g/b/d)	FCR
SB (g/kg)				
0	776	63.5	44.3	1.436
3	761	61.0	43.3	1.414
6	772	62.2	43.8	1.421
9	775	62.9	44.1	1.425
12	748	61.6	42.3	1.455
SEM	15.7	1.25	0.92	0.0181
Probabilities				
SB	0.679	0.646	0.570	0.568
L	0.399	0.623	0.299	0.411
Q	0.665	0.529	0.608	0.524
D	0.540	0.360	0.453	0.925
CV%	5.8	5.7	6.0	3.6

Abbreviations: BW, final body weight; CV%, coefficients of variation; D, deviations; FCR, feed conversion ratio; FI, daily feed intake; L, linear response; Q, quadratic response; SEM, pooled standard errors of mean; WG, daily weight gain.

4 | DISCUSSION

All birds remained healthy during the study. However, their weight was 19.5% below the Ross 308 female broiler target weight, possibly due to being fed mash rather than pelleted feed and being kept in small groups (Pirgozliev et al., 2016; Yang et al., 2020). This was not considered to be detrimental to the experimental objectives.

The determined chemical composition of SB agreed with previous reports (Biswas et al., 2010; Panaite et al., 2022; Tkacz et al., 2019; Tudor et al., 2019). It is recognised that SB product composition naturally varies, likely due to different climate and soil conditions, cultivars, geographical regions, processing and laboratory analysis techniques. The popularity of food with high antioxidant content is increasing; thus, the observed levels of total carotenoids (347 μg/g) in SB may be used to enhance standard poultry diets based on white maize (Ortiz et al., 2021).

Zhao et al. (2012) found that feeding SB flavones from leaves, included at 0.03%–0.10%, decreased daily FI without affecting the growth performance of broilers. Ma et al. (2015), reported the opposite, that supplementing SB phenols from berries from 0.05% to 0.10% increased FI and bird growth. Phenolic compounds in SB berries may vary between 0.06% to about 0.90% (Mihova & Ivanova, 2020; Tkacz et al., 2019). This suggests that at least in the diets with 9 and 12 g/kg SB supplementation in the reported study, we could expect dietary phenol levels similar to those by Zhao et al. (2012) and Ma et al. (2015), although no growth responses were observed. Interestingly, when incorporating 50 g/kg SB berries in broiler diets, Ben-Mahmoud et al. (2014) reported lower performance compared to the control. The reason for the observed inconsistency in bird response may be due to different dietary inclusion levels and the use of birds with different genotypes, different rearing systems, different experimental designs and feeding berries from different origins.

TABLE 6 Effect of dietary Sea Buckthorn (SB) inclusion level (g/kg) on the relative organ weight and the jejunal villus morphometry of broiler chickens.

Treatment	BW (g)	PG (%)	P (%)	SI (%)	C (%)	Liver (%)	VH (μm)	VW (μm)	CD (μm)	VH:CD	MLT (μm)
SB (g/kg)											
0	783	2.78	0.43	4.23	0.40	2.53	1581	124	114[a]	13.9	132
3	821	2.86	0.46	4.01	0.47	2.75	1599	127	115[a]	14.0	133
6	789	2.78	0.41	4.07	0.41	2.55	1637	116	123[b]	13.3	134
9	795	2.75	0.43	4.08	0.43	2.61	1568	131	119[ab]	13.2	144
12	754	2.79	0.41	4.24	0.37	2.71	1632	116	123[b]	13.3	134
SEM	–	0.079	0.020	0.115	0.026	0.095	19.8	4.8	2.0	0.25	3.6
Probabilities											
SB	–	0.875	0.404	0.518	0.089	0.393	0.075	0.126	0.004	0.113	0.154
L	–	0.723	0.341	0.812	0.398	0.457	0.266	0.451	0.001	0.022	0.227
Q	–	0.908	0.707	0.106	0.056	0.947	0.841	0.625	0.089	0.467	0.150
D		0.601	0.232	0.624	0.136	0.177	0.027	0.043	0.057	0.408	0.119
CV%		8.0	13.1	9.8	18.0	10.2	3.5	11.0	4.7	5.1	7.5

Abbreviations: BW, body weight of dissected birds; C, caeca; CD, crypt depth; CV%, coefficients of variation; D, deviations; L, linear response; MLT, muscle layer thickness; P, pancreas; PG, proventriculus and gizzard; Q, quadratic response; SEM, pooled standard errors of mean; SI, small intestine; VH, villus height; VW, villus width.

[a,b]Means within a column with no common superscripts differ significantly ($P < 0.05$).

TABLE 7 Effect of dietary Sea Buckthorn (SB) inclusion level (g/kg) on hepatic and blood antioxidant concentration of broiler chickens.

Treatment	Vit E (μg/g)	Q_{10} (μg/g)	Carotenoids (μg/g)	GSH-Px (U/g HB)
SB (g/kg)				
0	35	360	3.7	748
3	37	316	3.5	705
6	31	347	3.6	775
9	47	353	4.3	681
12	40	365	3.5	763
SEM	4.3	23.9	0.33	30.1
Probabilities				
SB	0.140	0.626	0.439	0.173
L	0.160	0.544	0.751	0.954
Q	0.772	0.330	0.570	0.452
D	0.084	0.538	0.197	0.059
CV%	31.8	19.4	25.4	11.6

Abbreviations: Carotenoids, hepatic carotenoids; CV%, coefficients of variation; D, deviations; GSH-Px, blood glutathione peroxidase; L, linear response; Q, quadratic response; Q_{10}, hepatic coenzyme Q_{10}; SEM, pooled standard errors of mean; Vit E, hepatic vitamin E.

The positive effects of SB on liver protection and treatment of liver diseases is well documented (Hsu et al., 2009; Solcan et al., 2013; Ting et al., 2011). Kalia et al. (2018) found that feeding flavones extracted from SB improved overall antioxidative status of broilers reared at high altitude under cold climate. Zhao et al. (2012) reported that dietary supplementation with SB protected/decreased the meat flavour loss in birds exposed to heat stress. Feeding SB berries or seed oil, increased GSH-Px in the blood serum of children suffering from diabetes (Nemes-Nagy et al., 2008) and hepatic GSH-Px in mice with carbon tetrachloride-induced hepatic damage (Hsu et al., 2009; Ting et al., 2011). However, the lack of response of hepatic antioxidants and blood plasma GSH-Px to dietary SB in our study may be explained by the good health of the birds and absence of disease challenge in the rearing environment.

In the reported study, the length of the jejunal villi of the birds was in the expected range for broilers of this age (Abdulla et al., 2016; Pirgozliev, Mansbridge, Rose, Mackenzie, et al., 2019). In an experiment with rats, Umirbekova et al. (2019) feeding SB berries in high-caloric fat diets mitigated some partial destructive changes in the small intestine linked to high-fat diets. Panaite et al. (2022) found an increase in duodenal and jejunal VH when feeding 20 g/kg SB meal to laying hens for 28 days. However, unlike the duodenum, there were

TABLE 8 Effect of dietary Sea Buckthorn (SB) inclusion level (g/kg) on the caecal production of short-chain fatty acids (mmol/L) in 21-day-old broiler chickens.

Treatment	Acetic acid	Butyric acid	Valeric acid	Propionic acid
SB (g/kg)				
0	378	56	6	48
3	286	57	6	36
6	256	48	4	44
9	220	40	3	40
12	188	26	1	35
SEM	52.2	9.7	1.1	5.6
Probabilities				
SB	0.132	0.162	0.031	0.412
L	0.012	0.017	0.002	0.203
Q	0.566	0.444	0.567	0.969
D	0.925	0.974	0.844	0.316
CV%	55.6	60.7	77.4	38.8

Abbreviations: CV%, coefficients of variation; D, deviations; L, linear response; Q, quadratic response; SEM, pooled standard errors of mean.

TABLE 9 Effect of Sea Buckthorn (SB) inclusion level (g/kg) on jejunal gene expression Log_{10} normalised copy number per reaction (normalised copy number per reaction), when fed to broiler chickens from 7 to 21 days age.

SB inclusion (g/kg)	CD40LG	IFNG	IL18	MUC2	OCLN
0	1.61 (41.1)	1.56 (38.5)	2.43 (279)	3.12 (1424)	2.88 (920)
3	1.63 (42.6)	1.69 (50.2)	2.62 (414)	3.34 (2261)	2.94 (914)
6	1.74 (55.5)	1.84 (72.8)	2.52 (340)	3.17 (1786)	3.05 (1631)
9	1.76 (60.1)	1.71 (56.6)	2.57 (383)	3.08 (1308)	3.01 (1208)
12	1.62 (42.2)	1.56 (40.6)	2.56 (390)	3.28 (1962)	3.23 (1814)
SEM	0.047	0.0954	0.066	0.111	0.1533
p Value					
SB	0.093	0.247	0.383	0.447	0.585
L	0.309	0.92	0.321	0.852	0.143
Q	0.033	0.034	0.338	0.94	0.764
D	0.245	0.756	0.321	0.179	0.808
CV%	5.6	11.4	5.2	6.9	10.1

Abbreviations: CV%, coefficients of variation; D, deviations; L, linear response; Q, quadratic response; SEM, pooled standard errors of mean.

no significant differences in CD and VH: CD in the jejunum (Panaite et al., 2022). Broilers in the present study fed up to 12 g/kg SB berries did not differ in VH, unlike in the study of Panaite et al. (2022), though there was a small increase in CD. The observed changes in CD due to SB in the reported study were relatively small to have biological significance.

Fermentation of dietary fibre by the microbiota in the distal part of the small intestine and ceca produces SCFA that has been related to overall gut health and small intestinal villus development (Thanh et al., 2009). Panaite et al. (2022) found that the inclusion of SB meal in laying hen diets increased the relative abundance of *Lactobacillus* spp., which could explain the linear reduction in acetic acid and increase in CD in the present study, as it is metabolised to lactic acid. The corresponding reduction in Bacteroidetes relative abundance (Panaite et al., 2022), may also account for the linear reduction in acetic acid in the present study confirming that SB addition may modulate intestinal morphology and hind gut bacterial fermentation in poultry.

In porcine IPEC-J2 cells, cytokine expression has been shown to be dose-dependent on SB polysaccharides (Zhao et al., 2020). We have also previously shown that supplemental phytogenic feed additives modulate CD40LG expression of caecal tonsil tissue (Pirgozliev, Mansbridge, Rose, Lillehoj, et al., 2019). In the present study, although there is not enough evidence that SB inclusion modulated immune cytokine expression (IFNG and CD40LG), there was a quadratic peak in these jejunal tissue cytokine expression at 6 and 9 g/kg diet, respectively. This peak in CD40LG and IFNG could indicate immunomodulation and a pro-inflammatory effect. Similarly, Patial et al. (2013) reported mild swelling and hyperplastic changes in the lining epithelial cells of villi in the jejunum of Japanese quail (*Coturnix*

japonica) supplemented with 2% powdered SB leaves compared to unsupplemented control-fed birds. In general, SB dietary inclusion in the present study did not significantly influence cytokine, mucin or tight junction gene expression. Based on the data, it is likely SB is safe for dietary inclusion at the levels studied but may not always be efficacious at improving the performance of healthy birds.

5 | CONCLUSIONS

It is concluded that the inclusion of dried SB berries at levels of between 3 and 12 g/kg diet, did not significantly affect broiler chicken growth performance, antioxidant status or intestinal health, with the exception of increased jejunal crypt depth and linear reductions in caecal concentrations of acetic, butyric and valeric acids. Research with higher dietary SB inclusion levels and/or SB extracts focusing on microbiome and immunomodulation may be warranted due to the inconsistency of study reports.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request, subject to restrictions and conditions.

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REFERENCES

Abdulla, J. M., Rose, S. P., Mackenzie, A. M., Ivanova, S. G., Staykova, G. P., & Pirgozliev, V. R. (2016). Nutritional value of raw and micronised field beans (*Vicia faba* L. var. *minor*) with and without enzyme supplementation containing tannase for growing chickens. *Archives of Animal Nutrition*, *70*(5), 350–363. https://doi.org/10.1080/1745039X.2016.1214344

Ben-Mahmoud, Z., Mohamed, M. S., Bláha, J., Lukešová, D., & Kunc, P. (2014). The effect of sea buckthorn (*Hippophae rhamnoides* L.) residues in compound feeds on the performance and skin color of broilers. *Indian Journal of Animal Research*, *48*(6), 548–555. https://doi.org/10.5958/0976-0555.2014.00030.2

Berndtson, W. E. (1991). A simple, rapid and reliable method for selecting or assessing the number of replicates for animal experiments. *Journal of Animal Science*, *69*(1), 67–76. https://doi.org/10.2527/1991.69167x

Biswas, A., Bharti, V. K., Acharya, S., Pawar, D. D., & Singh, S. B. (2010). Sea buckthorn: New feed opportunity for poultry in cold arid Ladakh region of India. *World's Poultry Science Journal*, *66*(4), 707–714. https://doi.org/10.1017/S004393391000067X

Diandong, H., Feng, G., Zaifu, L., Helland, T., Weixin, F., & Liping, C. (2016). Sea buckthorn (*Hippophae rhamnoides* L.) oil protects against chronic stress-induced inhibitory function of natural killer cells in rats. *International Journal of Immunopathology and Pharmacology*, *29*(1), 76–83. https://doi.org/10.1177/0394632015619939

Englyst, H. N., Quigley, M. E., & Hudson, G. J. (1994). Determination of dietary fibre as non-starch polysaccharides with gas–liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst*, *119*(7), 1497–1509. https://doi.org/10.1039/AN9941901497

Hsu, Y. W., Tsai, C. F., Chen, W. K., & Lu, F. J. (2009). Protective effects of seabuckthorn (*Hippophae rhamnoides* L.) seed oil against carbon tetrachloride-induced hepatotoxicity in mice. *Food and Chemical Toxicology*, *47*(9), 2281–2288. https://doi.org/10.1016/j.fct.2009.06.015

Kalia, S., Bharti, V. K., Giri, A., Kumar, B., Arora, A., & Balaje, S. S. (2018). *Hippophae rhamnoides* as novel phytogenic feed additive for broiler chickens at high altitude cold desert. *Scientific Reports*, *8*(1), 5954.

Karadas, F., Pirgozliev, V., Rose, S. P., Dimitrov, D., Oduguwa, O., & Bravo, D. (2014). Dietary essential oils improve the hepatic antioxidative status of broiler chickens. *British Poultry Science*, *55*(3), 329–334. https://doi.org/10.1080/00071668.2014.891098

Kumar, S., Ciraci, C., Redmond, S. B., Chuammitri, P., Andreasen, C. B., Palić, D., & Lamont, S. J. (2011). Immune response gene expression in spleens of diverse chicken lines fed dietary immunomodulators. *Poultry Science*, *90*(5), 1009–1013. https://doi.org/10.3382/ps.2010-01235

Ma, J. S., Chang, W. H., Liu, G. H., Zhang, S., Zheng, A. J., Li, Y., Xie, Q., Liu, Z. Y., & Cai, H. Y. (2015). Effects of flavones of sea buckthorn fruits on growth performance, carcass quality, fat deposition and lipometabolism for broilers. *Poultry Science*, *94*(11), 2641–2649. https://doi.org/10.3382/ps/pev250

Mihova, T., & Ivanova, P. (2020). Investigation of the biochemical composition of fruits from two genotypes sea buckthorn (*Hippophae rhamnoides* L.). *Scientific Papers. Series B. Horticulture*, *64*(2), 73–76.

Mishra, K. P., Chanda, S., Karan, D., Ganju, L., & Sawhney, R. C. (2008). Effect of Seabuckthorn (*Hippophae rhamnoides*) flavone on immune system: An *in-vitro* approach. *Phytotherapy Research*, *22*(11), 1490–1495. https://doi.org/10.1002/ptr.2518

Munyaka, P. M., Echeverry, H., Yitbarek, A., Camelo-Jaimes, G., Sharif, S., Guenter, W., House, J. D., & Rodriguez-Lecompte, J. C. (2012). Local and systemic innate immunity in broiler chickens supplemented with yeast-derived carbohydrates. *Poultry Science*, *91*(9), 2164–2172. https://doi.org/10.3382/ps.2012-02306

Nemes-Nagy, E., Szőcs-Molnár, T., Dunca, I., Balogh-Sămărghiţan, V., Hobai, Ş. T., Morar, R., Pusta, D. L., & Crăciun, E. C. (2008). Effect of a dietary supplement containing blueberry and sea buckthorn concentrate on antioxidant capacity in type 1 diabetic children. *Acta Physiologica Hungarica*, *95*(4), 383–393. https://doi.org/10.1556/APhysiol.95.2008.4.5

Ortiz, D., Lawson, T., Jarrett, R., Ring, A., Scoles, K. L., Hoverman, L., Rocheford, E., Karcher, D. M., & Rocheford, T. (2021). Biofortified orange corn increases xanthophyll density and yolk pigmentation in egg yolks from laying hens. *Poultry Science*, *100*(7), 101117. https://doi.org/10.1016/j.psj.2021.101117

Panaite, T. D., Vlaicu, P. A., Saracila, M., Cismileanu, A., Varzaru, I., Voicu, S. N., & Hermenean, A. (2022). Impact of watermelon rind and sea buckthorn meal on performance, blood parameters, and gut microbiota and morphology in laying hens. *Agriculture*, *12*(2), 177. https://doi.org/10.3390/agriculture12020177

Patial, V., Asrani, R. K., & Patil, R. D. (2013). Safety evaluation of seabuckthorn (*Hippophae rhamnoides*) leaves in Japanese quail. *Veterinary World*, *6*(9), 596–600. https://doi.org/10.5455/vetworld.2013.596-600

Percie du Sert, N. P., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Hurst, V., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., … Würbel, H. (2020). Reporting animal research: Explanation and elaboration for the ARRIVE Guidelines 2.0. *PLoS Biology*, *18*(7), e3000411. https://doi.org/10.1371/journal.pbio.3000411

Pirgozliev, V., Mansbridge, S., Whiting, I., Abdulla, J., Rose, S., & Mihova, T. (2022). Metabolisable energy of dried Sea buckthorn (*Hippophaes rhamnoides*) berries for broiler chickens. *Journal of Central European Agriculture*, *23*(3), 507–512. https://doi.org/10.5513/JCEA01/23.3.3589

Pirgozliev, V., Mansbridge, S. C., Rose, S. P., Lillehoj, H. S., & Bravo, D. (2019). Immune modulation, growth performance, and nutrient retention in broiler chickens fed a blend of phytogenic feed additives. *Poultry Science*, *98*(9), 3443–3449. https://doi.org/10.3382/ps/pey472

Pirgozliev, V., Mansbridge, S. C., Rose, S. P., Mackenzie, A. M., Beccaccia, A., Karadas, F., Ivanova, S. G., Staykova, G. P., Oluwatosin, O. O., & Bravo, D. (2019). Dietary essential oils improve feed efficiency and hepatic antioxidant content of broiler chickens. *Animal*, *13*(3), 502–508. https://doi.org/10.1017/S1751731118001520

Pirgozliev, V., Mirza, M. W., & Rose, S. P. (2016). Does the effect of pelleting depend on the wheat sample when fed to chickens? *Animal*, *10*(4), 571–577. https://doi.org/10.1017/S1751731115002311

Pirgozliev, V., Whiting, I. M., Mansbridge, S. C., Enchev, S., Rose, S. P., Kljak, K., Johnson, A. E., Drijfhout, F., Orczewska-Dudek, S., & Atanasov, A. G. (2021). Effect of rearing temperature on physiological measures and antioxidant status of broiler chickens fed stevia (*Stevia rebaudiana* B.) leaf meal and exogenous xylanase. *Current Research in Biotechnology*, *3*, 173–181. https://doi.org/10.1016/j.crbiot.2021.05.005

Solcan, C., Gogu, M., Floristean, V., Oprisan, B., & Solcan, G. (2013). The hepatoprotective effect of Sea buckthorn (*Hippophae rhamnoides*) berries on induced aflatoxin B1 poisoning in chickens 1. *Poultry Science*, *92*(4), 966–974. https://doi.org/10.3382/ps.2012-02572

Tanner, S. D., Baranov, V. I., & Bandura, D. R. (2002). Reaction cells and collision cells for ICP-MS: A tutorial review. *Spectrochimica Acta Part B*, *57*(9), 1361–1452. https://doi.org/10.1016/S0584-8547(02)00069-1

Thanh, N. T., Loh, T. C., Foo, H. L., Hair-Bejo, M., & Azhar, B. K. (2009). Effects of feeding metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. *British Poultry Science*, *50*(3), 298–306. https://doi.org/10.1080/00071660902873947

Ting, H., Hsu, Y., Tsai, C., Lu, F., Chou, M., & Chen, W. (2011). The *in vitro* and *in vivo* antioxidant properties of seabuckthorn (*Hippophae rhamnoides* L.) seed oil. *Food Chemistry*, *125*(2), 652–659. https://doi.org/10.1016/j.foodchem.2010.09.057

Tkacz, K., Wojdyło, A., Turkiewicz, I. P., Bobak, Ł., & Nowicka, P. (2019). Anti-oxidant and anti-enzymatic activities of sea buckthorn (*Hippophaë rhamnoides* L.) fruits modulated by chemical components. *Antioxidants*, *8*(12), 618. https://doi.org/10.3390/antiox8120618

Tudor, C., Bohn, T., Iddir, M., Dulf, F. V., Focşan, M., Rugină, D. O., & Pintea, A. (2019). Sea buckthorn oil as a valuable source of bioaccessible xanthophylls. *Nutrients*, *12*(1), 76. https://doi.org/10.3390/nu12010076

Umirbekova, O. B., Yessimsiitova, Z. B., Tleubekkyzy, P., Kozhamzharova, A. S., Assan, M. B., Tileshova, M. T., & Satybaldina, B. (2019). Morphofunctional study of the rat intestine in experiment. *Вестник Казахского Национального медицинского университета* (*Bulletin of the Kazakh National Medical University*), *2*, 149–151.

Whiting, I. M., Pirgozliev, V., Kljak, K., Orczewska-Dudek, S., Mansbridge, S. C., Rose, S. P., & Atanasov, A. G. (2022). Feeding dihydroquercetin in wheat-based diets to laying hens: Impact on egg production and quality. *British Poultry Science*, *63*, 735–741. https://doi.org/10.1080/00071668.2022.2090229

Woods, S. L., Rose, S. P., Whiting, I. M., Yovchev, D. G., Ionescu, C., Blanchard, A., & Pirgozliev, V. (2021). The effect of selenium source on the oxidative status and performance of broilers reared at standard and high ambient temperatures. *British Poultry Science*, *62*(2), 235–243. https://doi.org/10.1080/00071668.2020.1824292

Yang, Z., Pirgozliev, V. R., Rose, S. P., Woods, S., Yang, H. M., Wang, Z. Y., & Bedford, M. R. (2020). Effect of age on the relationship between metabolizable energy and digestible energy for broiler chickens. *Poultry Science*, *99*(1), 320–330. https://doi.org/10.3382/ps/pez495

Yeung, A. W. K., Tzvetkov, N. T., Zengin, G., Wang, D., Xu, S., Mitrović, G., Brnčić, M., Dall'Acqua, S., Pirgozliev, V., Kijjoa, A., Georgiev, M. I., & Atanasov, A. G. (2019). The berries on the top. *Journal of Berry Research*, *9*(1), 125–139. https://doi.org/10.3233/JBR-180357

Zhao, L., Li, M. Y., Su, S., Geng, T. T., & Sun, H. (2020). *Hippophae rhamnoides* L. polysaccharide enhances antioxidant enzyme activity, cytokine level, and related mRNA expression in intestinal porcine epithelial cells. *Canadian Journal of Animal Science*, *100*(1), 193–204. https://doi.org/10.1139/cjas-2019-0134

Zhao, W., Chen, X., Liu, H., Ouyang, W., Wang, M., & Li, Y. (2012). Effects of flavones from sea buckthorn leaves on growth performance and carcass quality of broilers. *Chinese Journal of Animal Nutrition*, *24*, 117–123.

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