



Prebiotic effect of *Ulva rigida* meal on the intestinal integrity and serum cholesterol and triglyceride content in broilers

Betsabé Cañedo-Castro¹ · Alejandra Piñón-Gimate¹ · Silvia Carrillo² · David Ramos³ · Margarita Casas-Valdez¹

Received: 16 August 2018 / Revised and accepted: 7 March 2019 / Published online: 21 March 2019
© Springer Nature B.V. 2019

Abstract

Marine algae contain large amounts of bioactive compounds and dietary fiber; thus, when used as feed for poultry, they could be an alternative to improve intestinal integrity and reduce lipid serum concentrations. Few studies have assessed the prebiotic properties of this marine resource. The objective of this study was to evaluate the prebiotic effects of different concentrations of the green alga *Ulva rigida* as feed additive to enhance the morphology of intestinal villi and reduce total cholesterol and triglyceride levels in chickens. One hundred and forty-one-day-old Arbor Acres broilers were randomized to one of four treatments: 0, 2, 4, and 6% *Ulva* meal, respectively, including seven replicates of five broilers each, in a completely randomized design. The assay was run for 6 weeks. Body weight gain and carcass percentage were not affected by the treatment, but feed intake, feed conversion ratio, and mortality showed significant differences ($p < 0.05$). Width, height, and contour length of intestinal villi were higher ($p < 0.05$) in all *U. rigida* meal treatments compared to the control group. The highest ($p < 0.05$) intestinal villus height and contour length were recorded with 2% *Ulva*. Serum total cholesterol and triglyceride levels were significantly lower in *Ulva* treatments vs. control ($p < 0.05$). The addition of *U. rigida* to broilers meal improved the growth of intestinal villi and reduced serum total cholesterol and triglyceride levels, thus confirming that it could be considered as a prebiotic that can enhance the broiler health.

Keywords Broilers · Cholesterol · Intestinal integrity · Prebiotic effects · Triglycerides · Chlorophyta · *Ulva rigida*

Introduction

Marine algae contain numerous compounds such as protein, minerals and vitamins, pigments, amino acids, fatty acids, complex polysaccharides, dietary fiber, and phenolic compounds that possess bioactive and/or nutraceutical properties. Thus, they may contribute to improve the health status of poultry by strengthening their immune system and reducing or modifying the microbial load in the intestine of chickens or hens (Evans and Critchely 2014; Kulshreshtha et al. 2014;

Qadri et al. 2019). Marine algae have also been shown to reduce serum cholesterol and triglyceride contents, reduce blood pressure, and promote a healthy digestion; they also have some antioxidant activity that could improve the health condition of poultry in commercial production processes (Raghavendran et al. 2005; Kulshreshtha et al. 2014; Li et al. 2018).

The use of prebiotics as growth promoters can be a suitable option in the poultry industry (Arce-Menocal et al. 2008; Buclaw 2016). The advantages of these products derive from their specific effects on the digestive tract, including modification of the intestinal flora, lowering of the mucosal turnover rate, and regulation of the intestinal immune system; these translate into direct benefits on growth rate, feed conversion efficiency, and bird health (Arce-Menocal et al. 2005).

However, few studies have explored the use of seaweeds as supplements of broilers feed (Makkar et al. 2016); the direct effect of seaweeds on intestinal morphology has been little investigated; studies addressing their influence on blood variables of broiler chicken are scarce. In these regards for brown seaweeds, Koth et al. (2005) included up to 4% *Undaria pinnatifida* to poultry feed, which relieved the inflammation

✉ Margarita Casas-Valdez
mcasav33@gmail.com

¹ Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Avenida Instituto Politécnico Nacional s/n, 23096 La Paz, B.C.S., Mexico

² Departamento de Nutrición Animal, Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán”, Vasco de Quiroga No. 15, Sección XVI, 14000 CDMX, Mexico

³ Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, 04510 CDMX, Mexico

resulting from the overstimulation of the innate immune system. El-Deek et al. (2011) observed that 2% *Sargassum* supplemented to feed led to significant increases in plasma high-density lipoprotein (HDL) and lower total cholesterol levels. Lee et al. (2005) supplemented the diet of broilers with 1, 2, and 4% *U. pinnatifida* and observed that algae stimulated the innate immune system of broilers. For their part, Evans and Critchely (2014) found that *Ascophyllum nodosum* increased growth performance when fed to broilers. In the case of red seaweeds, Bradbury et al. (2012) reported that because of the high calcium concentration of calcified red seaweeds when included in broilers feed the bone health status of chickens. With respect to green seaweeds, Ventura et al. (1994) studied the effect of feed supplemented with *Ulva rigida* at 10, 20, and 30% on chicken performance; these authors reported that this marine algae was harmful when included in the diet at levels above 10%. Sun et al. (2010) and Wang et al. (2013) observed better availability and use of nutrients, a positive effect on both feed intake and feed conversion ratio, and lower abdominal and subcutaneous fat, when broilers were fed a diet supplemented with 2% to 4% of *Enteromorpha prolifera* (Now *Ulva*). Abudabos et al. (2013) added 1% and 3% *Ulva lactuca* to broiler chicken diets, resulting in higher breast muscle yield and weight, lower abdominal fat, and lower serum concentration of lipids, cholesterol, and uric acid.

Species of the genus *Ulva* are abundant in coastal waters around the world. There are eight species belonging to this genus in Bahía de La Paz, Baja California Sur, Mexico (Cruz-Ayala et al. 2001; Águila-Ramírez et al. 2005). Some localities of the bay show optimum conditions for a high abundance of *Ulva* species all year round in this area (Chávez-Sánchez et al. 2017), given its subtropical location (Cervantes-Duarte et al. 2001). *Ulva rigida* is the most abundant species; it is present throughout the year, with the highest coverage and biomass per square meter during spring and summer (Chávez-Sánchez et al. 2018). Therefore, this marine algae could be harvested for use as a supplement in poultry feed.

Some important polysaccharide components that can be found in the species of the genus *Ulva* are ulvans. These are mainly composed of rhamnose, xylose, and guluronic and iduronic acids (Robic et al. 2009). Ulvans can be considered as ideal prebiotics due to the following properties: (a) selectivity by beneficial bacteria but not by pathogenic strains, (b) non-digestibility, and (c) fermentability, as substrates for intestinal microbiota (Kulshreshtha et al. 2014; Li et al. 2018).

Thus, the aim of this study was to evaluate the prebiotic effects of different concentrations of *U. rigida*, an abundant marine algae in the Gulf of California, as feed supplement that may improve the morphology of intestinal villi and contribute to lower cholesterol and triglyceride levels in broiler chicken.

Materials and methods

Ulva rigida plants were collected manually from the intertidal zone of beaches at Bahía de La Paz, Baja California Sur (BCS), Mexico. Whole plants were washed with sea water and fresh water to remove sand and epibionts. Afterwards, plants were sun-dried (80% shadow mesh) for 4 days and ground in a hammer mill (Jerza model L).

A 1-kg sample of algae was obtained by quartering, and the following chemical analyses were conducted, in triplicate, using standard methods (AOAC 2000): moisture (oven-drying at 60 °C to constant weight, method 976.05), ash content (ignition at 550 °C in an electrical furnace, method 923.03), crude fiber (Fibertec apparatus, method 962.09), ether extract (Soxhlet apparatus, method 920.39), nitrogen content (micro-Kjeldhal method 976.05), and protein content, calculated using a factor of 6.25. Gross energy was determined using a Parr bomb calorimeter. Samples were subjected to acid digestion for mineral content quantification. Spectrophotometry procedures (method 969.08) were used to determine Ca, Mg, Na, K, Fe, Zn, Pb, and Cu.

Experimental design A total of 140 1-day-old Arbor Acres broilers were allocated to one of four treatments, with seven replicates of five broilers each: 0, 2, 4, and 6% *Ulva* meal, respectively, using a completely randomized design in a 6-week experiment. In all treatments, feed and water were offered ad libitum.

Starter and finisher diets (Table 1) were formulated using the software Nutrition (version 3.2) to meet the nutrient requirements of Arbor Acres broilers. The *U. rigida* meal partially replaced corn grain, soybean, vegetable oil, and supplement; all treatment diets were isonitrogenous and isocaloric (NCR 1994).

The percentage of *Ulva* supplement was decided based on previous experiments conducted with *Sargassum* in the region (Carrillo et al. 2012) and *Ulva* species, such as Lee et al. (2005), Sun et al. (2010), and Wang et al. (2013), all of which showed a good performance at these concentrations, but also based on the experiment by Ventura et al. (1994), suggesting not to exceed 10% of algal supplement in the broiler feed.

Production parameters Body weight, food intake, and mortality were recorded weekly. The feed conversion ratio adjusted for mortality was estimated at the end of the experiment.

Carcass yield At the end of the experiment, four broilers from each replicate of the treatments ($n = 112$) of 42 days of age were randomly selected and slaughtered. After euthanasia, feathers, head, neck, shanks, liver, and intestine were removed, and the remaining carcass was weighed:

Table 1 Composition of starter (1–21 days) and finisher (22–42 days) experimental diets (%)

Ingredients	0% <i>Ulva</i>	2% <i>Ulva</i>	4% <i>Ulva</i>	6% <i>Ulva</i>
Starter				
<i>U. rigida</i>	0	2	4	6
Corn grain	55	54	52	49
Soybean	37	36	38	39
Vegetable oil	4	4	3	3
Premix ^a	4	4	3	3
Finisher				
<i>U. rigida</i>	0	2	4	6
Corn grain	63	62	60	56
Soybean	29	28	30	32
Vegetable oil	4	4	4	4
Premix ^a	4	4	2	2

^aPremix: vitamins per kg: 9,500,000 IU vit. A, 3,500,000 IU vit D, 17000 IU vit. E, 2400 g vit. K, 15.5 mg vit. B12, 1000 g vit. B1, 6.7 g vit. B2, 1.5 g vit. B6, 24.2 g niacin, 1 g folic acid, 125 mg biotin, 11.2 g pan-cal, 950 g choline chloride. Minerals (mg kg⁻¹): magnesium 67, zinc 67, iron 54, copper 4, iodine 0.6, selenium 1.4

Carcass yield (%) = (carcass weight/live weight) × 100.

Intestinal integrity After viscera were removed, 2-cm jejunum segments (1 cm after Meckel's diverticulum) were sectioned for each of the 112 broilers. These segments were briefly

washed in 0.1 M phosphate buffer solution (pH 7.3) and fixed in 12% formaldehyde for 24 h. Afterwards, three portions of each mid-jejunum segment were embedded in paraffin wax, sectioned (4 µm), and stained with hematoxylin-eosin (Hu et al. 2013). Morphological measurements of villus (width, height, and contour length) were taken with an upright microscope DM4 B (Leica DMC2900) equipped with a digital camera, using a 10× objective and a Dell Workstation or HP Software. Villus width is the horizontal distance between the walls of a single villus; height was measured as the vertical distance from tip to base; and contour length is the length of the line that delineates a single villus (Fig. 1).

Cholesterol and triglyceride quantification Before sacrifice, blood samples were collected from the radial vein of each of the 112 broilers, according to the Mexican standard NOM-069-200-1999. A 6.5- mL blood sample was collected in a 15-mL Falcon tube and centrifuged at 4500 rpm for 10 min; serum was separated, transferred to Eppendorf tubes, and stored at -70 °C until analyses. Samples were tested for (a) cholesterol, determined by enzymatic hydrolysis and oxidation (final point enzymatic method; Tietz 2006). The indicator was a quinoneimine dye formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Absorbance was read at 546 nm; (b) triglycerides, determined by enzymatic hydrolysis with lipases (GPO-PAP method; James et al. 2013). The indicator was a quinoneimine formed by hydrogen peroxide, 4-amino-phenazone, and 4-

Fig. 1 Measurement of intestinal villus in broilers fed different percentages of *Ulva* meal in the diet: **a** height, **b** width, **c** contour length

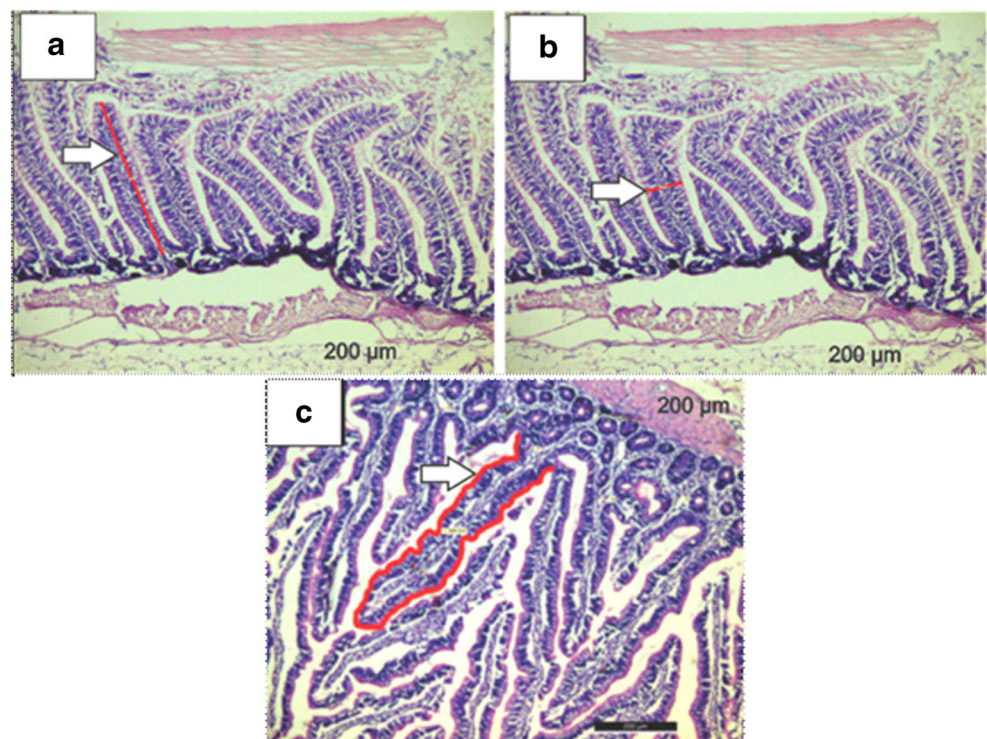


Table 2 Chemical composition of *Ulva rigida*

Nutrient content	
Moisture %	16.2 ± 0.03
Ash %	30.1 ± 0.16
Crude protein ($N \times 6.25$) %	8.7 ± 0.05
Nitrogen %	1.4 ± 0.01
Ether extract %	0.2 ± 0.03
Crude fiber %	4.2 ± 0.06
Nitrogen-free extract %	55.9
Gross energy Cal g ⁻¹	2212.3 ± 2.10
Calcium mg kg ⁻¹	11,778.83
Magnesium mg kg ⁻¹	20,916.53
Sodium mg kg ⁻¹	10,655.63
Potassium mg kg ⁻¹	8001.00
Lead mg kg ⁻¹	N.D.
Zinc mg kg ⁻¹	10.18
Copper mg kg ⁻¹	1.35
Iron mg kg ⁻¹	463

N.D. not detected, ± mean and standard deviation

chlorophenol, with peroxidase as catalyzer. Absorbance was read at 546 nm. All tests were carried out in triplicate using a Semi-auto Chemistry Analyzer, Model EKEM (Kontrol Lab).

Statistical analyses Each data set was tested for normality and homoscedasticity using the Kolmogorov-Sminov and Levene's tests, respectively (Zar 2010). Differences between data sets were analyzed through an analysis of variance (ANOVA) for a completely randomized design, and means were compared with the Tukey's test ($p < 0.05$) (Sall et al. 2007). All statistical analyses were run in the JMP® Start Statistics program, version 11.0.0.

Results

The chemical composition of the *Ulva rigida* meal used for this experiment showed a high ash content (30%), followed by crude protein (8%), crude fiber (4.2%), and a low ether extract content (0.2%). The minerals recorded at the highest concentration were magnesium with 20,916 mg kg⁻¹ calcium, 11,778 mg kg⁻¹ and 10,655 mg kg⁻¹ sodium (Table 2).

At the end of the experiment, no significant differences were found in body weight ($p > 0.05$); however, feed intake, feed conversion ratio, and mortality showed significant differences ($p < 0.05$) (Table 3). Feed intake was higher ($p < 0.05$) in chickens fed 4 and 6% of *Ulva*, whereas mortality was higher ($p < 0.05$) in the control and 6% *Ulva* treatments (Table 3). In relation to carcass weight and carcass yield, no significant differences were found among treatments (Table 3).

Intestinal villus growth was higher in all treatments with *U. rigida* meal (Fig. 2). Villus width, height, and contour length were significantly higher ($p < 0.05$) in all *U. rigida* meal treatments compared to the control. In the control (0% *Ulva*), intestinal villus width was 0.4 mm; in *U. rigida* meal treatments (2, 4, and 6% *Ulva*, respectively), it ranged from 0.6 to 0.7 mm. The highest ($p < 0.05$) value of intestinal villus height was recorded in the 2% *Ulva* treatment (1.6 mm), versus 1 mm in the control. Villus contour length was significantly higher ($p < 0.05$) in the 2% *Ulva* treatment (3.4 mm) vs. all other treatments; it was 2.8 mm in the 4 and 6% *Ulva* treatments and lowest (2 mm) in the control (0% *Ulva*) (Figs. 3 and 4).

Cholesterol concentration in broilers fed *U. rigida* meal was significantly lower ($p < 0.05$) vs the control diet. The 2% *Ulva* meal led to a 14% reduction in total cholesterol levels vs. control (Table 4). Moreover, triglyceride concentration in broilers was lower ($p < 0.05$) in all *U. rigida* meal treatments. The reduction in triglyceride concentration vs.

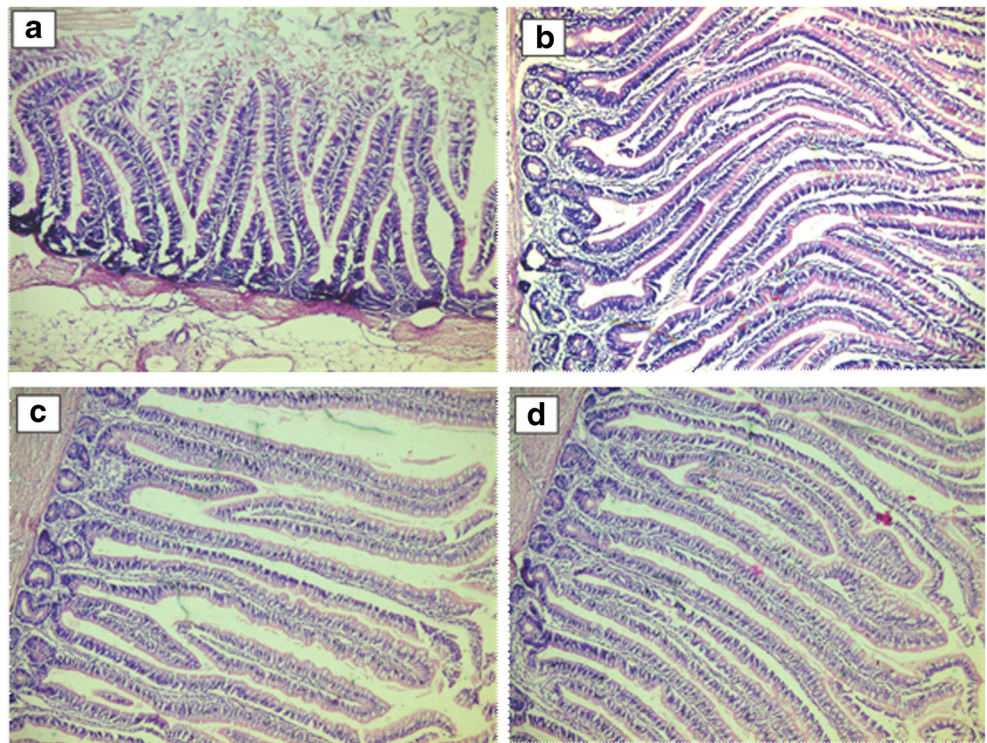
Table 3 Productive parameters and carcass yield of Arbor Acres broilers fed with different levels of *Ulva* inclusion in the diet

Treatments	Body weight (g)	Feed intake (g)	Feed conversion ratio	Mortality (%)	Carcass weight (g)	Carcass yield (%)
0% <i>Ulva</i>	2243	4098 ac	1.871 a	1.40	1552.42	74.7
2% <i>Ulva</i>	2262	4177 ac	1.881 a	0.35	1629.58	74.8
4% <i>Ulva</i>	2119	4205 bc	2.029 b	0.35	1481.25	73.8
6% <i>Ulva</i>	2257	4286 bd	1.941 a	1.20	1614.46	74.6
<i>P</i>	0.1266	< .0001	0.0388	0.49	0.052	0.4096
SEM	46.9	21.6	3.26	0.55	21.2	0.79

a,b,c Different letters in each column indicate significant difference ($p < 0.05$)

SEM standard error of the mean

Fig. 2 Intestinal villi in broilers fed different percentages of *Ulva* meal in the diet: **a** 0% *Ulva*, **b** 2% *Ulva*, **c** 4% *Ulva*, **d** 6% *Ulva*



the 0% *Ulva* group was 28% (2% *Ulva*), 17% (4% *Ulva*), and 15% (6% *Ulva*) (Table 4).

Discussion

Ash content, crude protein, crude fiber, and ether extract recorded in *Ulva rigida* meal were lower than the values reported by Carrillo et al. (2002), and within the ranges reported by Aguilera-Morales et al. (2005) for *Enteromorpha* (now *Ulva*) collected in the same locality. Proximal chemical analyses of *U. rigida* from other coastal waters (Dardanelles, Turkey; Chilka Lake, India; La Coruña, Spain; Gulf of Galez,

Tunisia) recorded the following ranges: 4.8–21.2% protein, 0.09–12% lipids, 11–52% ash (Foster and Hodgson 1998; Taboada et al. 2010; Frikha et al. 2011; Gopal and Pal 2011; Irkin and Erdugan 2014). The differences in nutrient content are likely because seaweeds have an extraordinary capacity to accumulate elements present in water (Cabrita et al. 2016); therefore, seasonal, annual, and geographic environmental variability (temperature, salinity, irradiance, nutrients) could influence nutrient content in seaweeds (Marinho-Sorino et al. 2006).

Constant *Ulva* concentrations were used (2, 4, and 6%) in the starter and finisher diets. As the proportion of *U. rigida* in the meal increased, soybean content was increased and corn grain decreased to compensate for the nutritional requirements

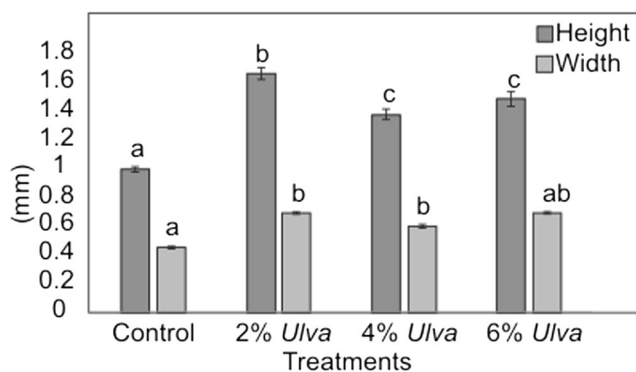


Fig. 3 Height and width of intestinal villi at day 42 of the experiment in broilers fed different percentages of *Ulva* meal in the diet. a,b,c Different letters indicate a significant difference ($p < 0.05$)

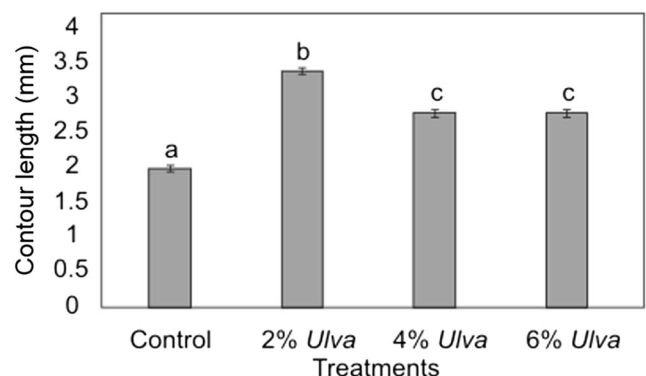


Fig. 4 Contour length of intestinal villi at day 42 of the experiment in broilers fed different percentages of *Ulva* meal in the diet. a,b,c Different letters indicate a significant difference ($p < 0.05$)

for broilers, since *Ulva* has a low protein and a high crude fiber content.

As for broiler weight, a 32% gain was recorded in the starter period (days 1–21), and a 68% increase in the finisher period (days 22–42); the likely cause was that during the final period, broilers have a fully developed digestive system; therefore, the energy consumed is directly allocated to body weight (Cuervo et al. 2002; Rebolé et al. 2010).

In relation to the production parameters, differences in body weight (BW) were not statistically significant; in contrast, significant differences between treatments ($p < 0.05$) were recorded for feed intake, feed conversion ratio (FCR), and mortality. Although differences were expected, it is worth mentioning that body weight did not decrease with the supplemented treatments, as discussed by Abudabos et al. (2013). These authors included 1% and 3% of *Ulva lactuca* in the diet and found no significant differences, which could be related to the low seaweed concentration used, and since no loss was observed, they suggest that this finding may indicate the absence of toxic or anti-nutritional effects caused by *Ulva*. Also, other studies have found no significant differences in BW and FCR; for example, Cortes-Cuevas et al. (2000), Rebolé et al. (2010), and Chávez et al. (2016), who used probiotics and prebiotics in different concentrations.

In regard to body weight, it is important to note that this study obtained a body weight of 1.6 kg in 5 weeks, i.e., 1 week earlier than for commercial production (Ferrini et al. 2010); for commercial purposes, this could translate into lower meal expenses. Feed intake increased with 4% and 6% *Ulva* meal, which could have been related to the attractant properties of this algae (Cruz-Suarez et al. 2000).

In all treatments, carcass yield ranged from 73.8 to 74.8% with no significant differences between treatments; this figure was higher than those previously reported. Some examples include poultry farms, with an average of 70% (SAGARPA 2016); studies that used diets supplemented with symbiotic and probiotics (66.7% and 59.5%, respectively) (Awad et al. 2009); or poultry fed diets supplemented with 1% *Ulva* (70.7%) and 3% *Ulva* (71.3%) (Abudabos et al. 2013). Nonetheless, our values were lower than those obtained by Itzá-Ortiz et al. (2008) (76.8–77.8%) for diets including different energy sources, and those reported by Jaramillo (2012) using Fortifeed, a commercial probiotic (77.4%). El-Deek et al. (2011) reported a non-significant effect on carcass characteristics using different concentrations of *Sargassum* meal in finisher broiler diets.

Some experiments have investigated the effect of prebiotics on the increase of intestinal villi in broilers. Chávez et al. (2016) used *Lactobacillus casei* and *L. acidophilus* in broiler diet and found a villus length of 939 μm compared to 754 μm in the control group. Markovic et al. (2009) supplemented broiler feed with BIO-MOS®, a prebiotic derived from the cell wall of *Saccharomyces cerevisiae*, and found a significant

Table 4 Cholesterol and triglyceride concentration in Arbor Acres broilers fed different percentages of *Ulva* meal in the diet

Treatments	Cholesterol (mg dL ⁻¹)	Triglycerides (mg dL ⁻¹)
0% <i>Ulva</i>	140.1 a	93.3 a
2% <i>Ulva</i>	120.7 b (14%)	67.3 b (28%)
4% <i>Ulva</i>	125.6 b (10%)	77.0 c (17%)
6% <i>Ulva</i>	126.7 b (10%)	78.0 c (15%)
<i>p</i>	< .0001	< .0001
SEM	13.86	11.08

Different lowercase letters in each column indicate a significant difference ($p < 0.05$)

SEM standard error of the mean

Values in parentheses show the percentage of cholesterol and triglyceride reduction vs. control

increase in villus height (1013 μm vs 901 μm) and width (108 μm vs 94 μm). Arce-Menocal et al. (2008) added *S. cerevisiae* cell walls to poultry feed and recorded a villus width of 398 μm versus 264 μm in the control after 21 days. Rehman et al. (2007) used inulin as supplement and found that intestinal villus length was greater vs. control. In the present study, in broilers fed a 2% *U. rigida* meal, villus width, height, and contour length were 0.69 mm, 1.66 mm, and 3.4 mm, respectively, versus 0.45 mm, 1 mm, and 2.0 mm, respectively, in the control treatment. The structure of the intestinal mucosa affects its function, as higher villi increases the surface area and facilitate an efficient nutrient absorption (Rehman et al. 2007).

The higher values of intestinal villus metrics observed in all *U. rigida* meal treatments suggest that *Ulva* algae produce a nutraceutical effect on intestinal integrity. This is relevant because the increased length of intestinal villi produced both a larger intestinal surface area and increased activity of the brush border enzymes, leading to larger surface area for absorption and higher digestive capacity, respectively (Velasco et al. 2010); these features may also have enhanced nutrient uptake by increasing the absorption of fatty acids, amino acids, and glucose (Arce-Menocal et al. 2008). Furthermore, the *U. rigida* meal functioned as a prebiotic that may have stimulated the growth of beneficial intestinal bacteria, thereby reducing intestinal turnover and, consequently, lowering energy and protein expenditure (Xu et al. 2003).

Other researchers have found that n-6 and n-3 unsaturated fatty acids in the diet promoted intestinal villus growth in terms of both villus number and length (Itzá-Ortiz et al. 2008). *Enteromorpha* spp. (now *Ulva*) from the La Paz waterfront contains both n-3 (10.38 g (100 g)⁻¹) and n-6 (10.9 g (100 g)⁻¹) fatty acids (Aguilera-Morales et al. 2005), which may also promote villus growth.

The human consumption of food of certain characteristics has stimulated the search of higher-quality commercial

poultry, particularly as low cholesterol and triglyceride contents are highly desirable in food for human consumption. Osorio et al. (2012) reported that Ross 308 and Cobb broilers fed commercial diets showed a cholesterol concentration of 136 and 132 mg dL⁻¹, respectively, both higher compared to the 120–126 mg dL⁻¹ obtained in this study using *U. rigida* meal diets.

A significant reduction in cholesterol (14–10%) and triglycerides (28–15%) was observed with all *U. rigida* meal diets. This effect of *Ulva* is consistent with the results by Abudabos et al. (2013), who reported a cholesterol reduction of 19 and 20% in broilers fed 1 and 3% *U. lactuca*, respectively.

El-Deek et al. (2011) found that the addition of raw *Sargassum* to broiler finisher diets led to lower plasma cholesterol levels. Carrillo et al. (2012) demonstrated the effect of *Sargassum* spp. on the reduction of cholesterol concentration in eggs 26%. These authors attributed this performance to the effect of some chemical compounds of the algae, such as sterols, polysaccharides (soluble fiber), poly-unsaturated fatty acids, and minerals. Taboada et al. (2010) report that *U. rigida* contains a high percentage of soluble fiber, which has been correlated with hypocholesterolemic effects.

In other studies, Hassan et al. (2011) evaluated the effect of sulfate polysaccharides extracted from *U. lactuca* on serum lipid parameters in albino rats fed a cholesterol-rich diet. These authors found that the sulfated polysaccharide extract induced a significant decrease in total serum lipids (–61%), total cholesterol (–49%), triglycerides (–66%), and LDL-cholesterol (–93%). They concluded that the decreased hypercholesterolemia by the *U. lactuca* extract was likely due to the increased activity of antioxidant enzymes (CAT, GSH-Px, and SOD), non-enzymes (GSH and T. thiol), and the lipid-limiting peroxidation process.

The polysaccharide extract composition of *U. lactuca* included rhamnose, xylose, glucuronic and galacturonic acids, galactose, glucose, arabinose, and mannose (Hassan et al. 2011). According to Robic et al. (2009), the first three polysaccharides are constituents of ulvans, which can be considered as prebiotics that may improve poultry health (Kulshreshtha et al. 2014; Li et al. 2018), as we observed in our experiment by the addition of *Ulva* in the diets.

Conclusion

The supplementation of broiler meal with *Ulva rigida* contributed to promote and improve the growth of intestinal villi; it also led to lower serum levels of total cholesterol and triglycerides in broiler chicken, thus confirming that it can be considered as a prebiotic that enhances the health condition of broilers and enhancing the importance of studies where intestinal integrity and serum parameters could be measured.

Acknowledgments The authors are grateful to Efraín Flores Montaña for his help in field sampling, and to Diego Armando Falcón Vidal for his collaboration during the experiment. Alma Ribera Camacho assisted in the histological analysis; María Elena Sánchez and Diana Fischer provided editorial services. Thanks also to the person in charge of the Poultry Area of the Posta Zootécnica at the Universidad Autónoma de Baja California Sur for facilitating the conduct of the experiment. Casas-Valdez and Piñón-Gimate authors thank Estímulo al Desempeño de la Investigación (EDI), Comisión de Fomento a las Actividades Académicas (COFAA), and Cañedo-Castro scholarship granted by CONACYT and Beca al Estímulo Institucional de Formación de Investigadores (BEIFI), Instituto Politécnico Nacional (IPN).

Funding information This study was funded by the project Instituto Politécnico Nacional-SIP20161094.

Compliance with ethical standards

The experiment was run in compliance with the Mexican standard NOM-069-200-1999. Also, the experimental procedures were performed in accordance with the Guidelines and Rules for Animal Experimentation and the Animal Ethics Committee, Universidad Autónoma de Baja California Sur, Mexico.

References

- Abudabos AM, Okab AB, Aljumaah RS, Samara EM, Abdoun KA, Al-Haidary AA (2013) Nutritional value of green seaweed (*Ulva lactuca*) for broiler chickens. Ital J Anim Sci 12:177–181
- Águila-Ramírez R, Casas-Valdez M, Hernández-Guerrero CJ, Marín-Alvarez A (2005) Biomasa de *Ulva* spp. (Chlorophyta) en tres localidades del Malecón de La Paz, Baja California Sur, México. Rev Biol Mar Ocean 40:55–61
- Aguilera-Morales M, Casas-Valdez M, Carrillo-Domínguez S, González-Acosta B, Pérez-Gil F (2005) Chemical composition and microbiological assays of marine algae *Enteromorpha* spp., as a potential food source. J Compos Anal 18:79–88
- AOAC (2000) Official methods of analysis. AOAC International, Washington, D.C.
- Arce-Menocal J, Ávila-González E, López C, García EA, García GF (2005) Efecto de paredes celulares (*Saccharomyces cerevisiae*) en el alimento de pollo de engorda sobre los parámetros productivos. Téc Pecu Méx 43:155–162
- Arce-Menocal J, Ávila-González E, López C (2008) Comportamiento productivo y cambios morfológicos en vellosidades intestinales del pollo en engorda a 21 días de edad con el uso de paredes celulares de *Saccharomyces cerevisiae*. Vet Mex 39:223–228
- Awad WA, Ghareeb K, Abdel-Raheem S, Böhm J (2009) Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult Sci 88:49–56
- Bradbury EJ, Wilkinson SJ, Cronin GM, Walk CL, Cowieson AJ (2012) The effect of marine calcium source on broiler leg integrity. In: Proc 23rd Annual Australian Poultry Science Symposium, Sydney NSW, Australia 19–22 February pp 85–88
- Buclaw M (2016) The use of inulin in poultry feeding: a review. J Anim Physiol Anim Nutr 100:1015–1022
- Cabrita ARJ, Maia MRG, Oliveira HM, Sousa-Pinto I, Almeida AA, Pinto E, Fonseca AJM (2016) Tracing seaweeds as mineral sources for farm-animals. J Appl Phycol 28:3135–3150
- Carrillo S, Casas-Valdez M, Ramos F, Pérez-Gil F, Sánchez-Rodríguez I (2002) Algas Marinas de Baja California Sur, México: Valor nutricional. Arch Latinoam Nutr 52:400–405

- Carrillo S, Bahena A, Casas-Valdez M, Carranco M, Calvo C, Ávila E, Pérez-Gil F (2012) The alga *Sargassum* spp. as alternative to reduce egg cholesterol content. *Cuban J Agri Sci* 46:181–186
- Cervantes-Duarte R, Aguirre-Bahena F, Reyes-Salinas A, Valdez-Holguín JE (2001) Caracterización hidrológica de una laguna costera de Baja California Sur, México. *Oceanides* 16:93–105
- Chávez S, García-Martínez J, Delgado-Ramos L, Pérez-Ortín JE (2016) The importance of controlling mRNA turnover during cell proliferation. *Curr Genet* 62:701–710
- Chávez-Sánchez T, Piñón-Gimate A, Serviere-Zaragoza E, Sánchez-González A, Hernández-Carmona G, Casas-Valdez M (2017) Recruitment in *Ulva* blooms in relation to temperature, salinity and nutrients in a subtropical bay of the Gulf of California. *Bot Mar* 60:257–270
- Chávez-Sánchez T, Piñón-Gimate A, Melton JT III, López-Bautista JM, Casas-Valdez M (2018) *Ulva* blooms in the southwestern gulf of California: reproduction and biomass. *Estuar Coast Shelf Sci* 200:202–211
- Cortes-Cuevas A, Ávila GE, Casaubon HT, Carrillo DS (2000) El efecto del *Bacillus toyoi* sobre el comportamiento productivo en pollos de engorda. *Vet Mex* 31:301–308
- Cruz-Ayala MB, Núñez-López RA, López GE (2001) Seaweeds in the southern gulf of California. *Bot Mar* 44:187–197
- Cruz-Suarez LE, Ricque-Marie D, Tapia-Salazar M, Guajardo-Barbosa C (2000) Uso de la harina de kelp (*Macrocystis pyrifera*) en alimentos para camarón. In: Cruz-Suarez LE, Ricque-Marie D, Tapia-Salazar M, Olvera-Novoa MA, Civera-Cerecedo R (eds) *Avances en Nutrición Acuicola V. Memorias del V Simposio Internacional de Nutrición Acuicola*, pp 227–266
- Cuervo M, Gómez C, Romero H (2002) Efecto de la utilización de un suplemento nutricional hidratado en pollos de engorde recién nacidos. *Rev Colombiana Cien Pecuarias* 15:319–329
- El-Deek AA, Al-Harhi MA, Abdalla AA, Elbanoby MM (2011) The use of brown algae meal in finisher broiler diets. *Egypt Poult Sci J* 31:767–781
- Evans FD, Critchley AT (2014) Seaweeds for animal production use. *J Appl Phycol* 26:891–899
- Ferrini G, Manzanilla EG, Menoyo D, Esteve-García E, Baucells MD, Barroeta AC (2010) Effects of dietary n-3 fatty acids in fat metabolism and thyroid hormone levels when compared to dietary saturated fatty acids in chickens. *Livestock Sci* 131:287–291
- Foster GG, Hodgson AN (1998) Consumption and apparent dry matter digestibility of six intertidal macroalgae by *Turbo sarmaticus* (Mollusca:Vegigastropoda: Turbinidae). *Aquaculture* 167:211–227
- Frikha F, Kammoun N, Hammami N, Mchirgui RA, Belbahri L, Gargouri Y, Miled N, Ben-Rebah F (2011) Chemical composition and some biological activities of marine algae collected in Tunisia. *Cienc Mar* 37:113–124
- Gopal GS, Pal R (2011) Biochemical composition and lipid characterization of marine green alga *Ulva rigida*- a nutritional approach. *J Algal Biomass Utiln* 2:10–13
- Hassan S, El-Twab SA, Hetta M, Mahmoud B (2011) Improvement of lipid profile and antioxidant of hypercholesterolemic albino rats by polysaccharides extracted from the green alga *Ulva lactuca* Linnaeus. *Saudi J Biol Sci* 18:333–340
- Hu X, Wang T, Li W, Jin F, Wang L (2013) Effects of NS *Lactobacillus* strains on lipid metabolism of rats fed a high-cholesterol diet. *Lipids Health Dis* 12:67
- Irkin LC, Erdugan H (2014) Chemical composition of *Ulva rigida* C. Agardh from the Canakkale Strait (Dardanelles), Turkey. *J Black Sea/Mediterr Environ* 20:114–121
- Itzá-Ortiz MF, López-Coello C, Ávila-González E, Gómez-Rosales S, Arce-Menocal J, Velásquez-Madrado PA (2008) Efecto de la fuente energética y el nivel de energía sobre la longitud de las vellosidades intestinales, la respuesta inmune y el rendimiento productivo en los pollos de engorda. *Vet Mex* 39:357–376
- James O, Godwin E, Otini I (2013) *Ulvaria chamae* (Annonaceae) plant extract neutralizes some biological effects of *Naja nigricollis* snake venom in rats. *Br J Pharmacol Toxicol* 4:41–50
- Jaramillo ÁH (2012) Evaluación de la mezcla de un ácido orgánico y un prebiótico en los parámetros productivos y alométricos de pollos de engorde con alimentación controlada. *Rev Colombiana Cienc Anim* 5:52–66
- Koth TS, Im JT, Park IK, Lee HJ, Choi DY, Choi CJ, Lee HG, Choi YJ (2005) Effect of dietary brown seaweed levels on the protein and energy metabolism in broiler chicks activated acute phase response. *J Anim Sci Tech (Kor)* 47:379–390
- Kulshreshtha G, Rathgeber B, Stratton G, Thomas N, Evans F, Critchley A, Hafting J, Prithiviraj B (2014) Immunology, health, and disease: feed supplementation with red seaweeds, *Chondrus crispus* and *Sarcodiotheca gaudichaudii*, affects performance, egg quality, and gut microbiota of layer hens. *Poult Sci* 93:2991–3001
- Lee W, Koh E, Woh J, Kim M, Park J, Lee K (2005) Obesity: the role of hypothalamic AMP-activated protein kinase in body weight regulation. *Int J Biochem Cell Biol* 37:2254–2259
- Li Q, Luo J, Wang C, Tai W, Wang H, Zhang X, Liu K, Jia Y, Lyv X, Wang L, He H (2018) Ulvan extracted from green seaweeds as new natural additives in diets for laying hens. *J Appl Phycol* 30:2017–2027
- Makkar HPS, Tran G, Heuzé V, Giger-Reverdin S, Lessire M, Lebas F, Ankers P (2016) Seaweeds for livestock diets: a review. *Anim Feed Sci Technol* 212:1–17
- Marinho-Sorino E, Fonseca PC, Carneiro MAA, Moreira WSC (2006) Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresour Technol* 97:2402–2406
- Markovic R, Šefer D, Krstic M, Petrujkic B (2009) Effect of different growth promoters on broiler performance and gut morphology. *Arch Med Vet* 41:163–169
- NCR (1994) Nutrient requirements of poultry, 9th edn. National Academic Press, Washington, DC
- Osorio JH, Flórez JD, Pérez JE (2012) Evaluación de los métodos directo, precipitado y Friedewald para la cuantificación de colesterol LDL y HDL en pollos de engorde. *Rev Med Vet* 24:85–90
- Qadri SSN, Biswas A, Mandal AB, Kumawat M, Saxena R, Nasir AM (2019) Production performance, immune response and carcass traits of broiler chickens fed diet incorporated with *Kappaphycus alvarezii*. *J Appl Phycol* 31:753–760
- Raghavendran HRB, Sathivel A, Devaki T (2005) Effect of *Sargassum polycystum* (Phaeophyceae)-sulphated polysaccharide extract against acetaminophen-induced hyperlipidemia during toxic hepatitis in experimental rats. *Molec Cell Biochem* 276:89–96
- Rebolé A, Ortiz LT, Rodríguez M, Alzueta C, Treviño J, Velasco S (2010) Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat-and barley-based diet. *Poult Sci* 89:276–286
- Rehman H, Böhm J, Zentek J (2007) Effects of differentially fermentable carbohydrates on the microbial fermentation profile of the gastrointestinal tract of broilers. *J Anim Physiol Anim Nutr* 92:471–480
- Robic A, Bertrand D, Sassi JF, Lerat Y, Lahaye M (2009) Determination of the chemical composition of ulvan, a cell wall polysaccharide from *Ulva* spp. (Ulvales, Chlorophyta) by FT-IR and chemometrics. *J Appl Phycol* 21:451–456
- SAGARPA (2016) 4to Informe de Labores 2015-2016. Secretaría de Agricultura, Ganadería, Pesca y Desarrollo Alimentario, México 154 pp
- Sall J, Lee C, Lehman A (2007) JMP® star statistic: a guide to statistic and data analysis using JMP®, 4th edn. SAS Institute, Cary
- Sun J, Song HL, Zhao J, Xiao Y, Qi R, Lin YT (2010) Effects of different dietary levels of *Enteromorpha prolifera* on nutrient availability and digestive enzyme activities of broiler chickens. *Chin J Anim Nutr* 22:1658–1664

- Taboada C, Millán R, Míguez I (2010) Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. J Sci Food Agric 90:445–449
- Tietz N (2006) Clinical guide to laboratory test. In: Tietz N (ed) A Guide, 4th edn. WB Saunders Co, Philadelphia
- Velasco S, Rodríguez ML, Alzuela MC, Rebolé A, Ortiz LT (2010) Los prebióticos tipo inulina en alimentación aviar. I: Características y efectos a nivel intestinal. Rev Complutense Cien Vet 4:87–104
- Ventura MR, Castañón JIR, McNab JM (1994) Nutritional value of seaweed (*Ulva rigida*) for poultry. Anim Feed Sci Technol 49:87–92
- Wang SB, Shi XP, Zhou CF, Lin YT (2013) *Enteromorpha prolifera*: effects on performance, carcass quality and small intestinal digestive enzyme activities of broilers. Chin J Anim Nutr 25:1332–1337
- Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ (2003) Effects of dietary fructo oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci 82:1030–1036
- Zar JH (2010) Biostatistical analysis, 5th edn. Prentice-Hall Inc, Upper Saddle River

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.