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# Effect of marine sulfurized polysaccharides as immunomodulators of the response to vaccination in broilers

Efecto de los polisacáridos sulfurados marinos como inmunomoduladores de la respuesta ante la vacunación en pollo de engorda

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**Abstract**

One of the obstacles poultry producers face is the mutation suffered by pathogens that complicate the combat against them; due to this, there are considered alternatives to improve productive and immunological parameters. The present study evaluated feed intake, weight gain, feed conversion, Newcastle antibody titers, and mortality in broilers, adding their diet with sulfated polysaccharides extracted from seaweed (SP). The experiment consisted of four treatments with six repetitions, each one with 25 animals of Cobb genetics. The treatments were: control with vaccination, control with SP, and SP with the vaccine. The food was weighed daily, the rejections and the birds were weighed weekly, blood samples were taken at days 9, 21, 28, 35, and 42 of life to determine antibodies to Newcastle. The control group SP obtained the best food conversion with 1.83 (P <0.05). The vaccine treatments with SP and vaccine generated the highest amount of antibodies (P <0.05). The SP product at the dose used did not increase the productive or immunological parameters, so it would be essential to carry out another study with different quantities of product inclusion.
**Keywords:** polysaccharides, antibodies, Newcastle.

**Resumen**

Uno de los obstáculos que enfrentan los productores de aves, es la mutación que sufren los patógenos y complica su combate, debido a esto se buscan alternativas para mejorar los parámetros productivos e inmunológicos. El objetivo fue evaluar el consumo de alimento, ganancia de peso, conversión alimenticia, títulos de anticuerpos para Newcastle y mortalidad en pollos de engorda adicionado en su dieta con polisacáridos sulfatados extraídos de algas marinas (PS). El experimento se dividió en cuatro tratamientos con seis repeticiones cada uno y 25 pollos cada una, los tratamientos consistieron en un control, uno con vacunación, uno control con PS y PS con vacuna, se pesó el alimento diariamente, los rechazos y las aves fueron pesadas semanalmente, se tomaron muestras de sangre a los días 9, 21, 28, 35 y 42 de vida, para determinación de anticuerpos para Newcastle. La mejor conversión alimenticia fue de 1.83 (P < 0.05) del grupo control PS. Los tratamientos vacuna con PS y vacuna generaron la mayor cantidad de anticuerpos (P < 0.05). El producto PS a la dosis utilizada no demostró aumentar los parámetros productivos ni inmunológicos, por lo cual sería importante realizar otro estudio con diferentes dosis de inclusión del producto.
**Palabras claves:** polisacáridos, anticuerpos, Newcastle.

## INTRODUCTION

Last decade, zoonotic viral diseases with high mortality have emerged, among these pathogens are the paramyxoviruses, to which Newcastle disease belongs (Xu *et al*., 2013). This continues to be of great importance in the poultry industry due to its worldwide distribution, high virulence and economic repercussions it entails. The disease transmission has been prevented by vaccination; however, this method alone has not been efficient, since application methods of the vaccine and the cold chain maintenance must be considered, as well as different strains and variants present in the field, age of application, control of wild birds and the passive immunity that this causes, with a lower reaction to the vaccines applied. In the above lies the importance of searching for alternatives to generate a more efficient protection against these viruses (Darrell *et al*., 2012; Xu *et al*., 2013; Dimitrov *et al*., 2017).

Multiple biological activities of sulfated polysaccharides have been described, within which antiviral, anticancer, antioxidant and anticoagulant activity are mentioned. The antiviral activity of polysaccharides was first described in 1958, from that date to the present day a large number of sulfated polysaccharides have been found, either synthetic or natural; both with antiviral activity (Xu *et al*., 2013). Among these properties, sulfated polysaccharides have shown the ability to inhibit paramyxovirus infections, improve the survival rate against Newcastle disease by almost 20 % and allow blocking infections with high viral load so that possible treatments can be established (Xu *et al*., 2013; Xu *et al*., 2015).

The aim of the present work was to evaluate the effect of marine sulfur polysaccharides, obtained from algae of the genus *Ulva*, as a natural alternative that allows the efficiency of bird immune system before vaccination, and that in turn favors the increase of productive parameters.

## MATERIAL AND METHODS

The present work was carried out in the broiler house of the Animal Production Department of the University Center of Biological and Agricultural Sciences of the Universidad de Guadalajara. Its location is Camino Ramón Padilla Sánchez No. 2100 Nextipac, Zapopan, Jalisco, Mexico; with coordinates 20°74'59.05", North Latitude and 103°50'96.38" West Longitude, and an altitude of 1670 m a.s.l (INEGI, 2020).

600 broilers of Cobb genetic line were used, 300 males and 300 females, one day old and vaccinated against Marek's disease. Birds were distributed in 4 treatments with 6 replicates of 25 birds; 1: control treatment without vaccine (C), 2: control treatment with polysaccharides (PC), 3: treatment with vaccine without polysaccharides (V) and 4: treatment with vaccine and polysaccharides (VP). The polysaccharides used were Olmix©

brand and they were used at a dose of 45 g per 500 L of water on days 2, 3, 7, 9, 10, 19, 21, 22, 34, 36 and 37.
Birds were housed in a house distributed in 2.5 $m^2$ pens; three pens for females and three pens for males for each treatment. Freeze-dried Lukert strain gumboro vaccine was administered to four treatments at 5 and 14 days of age; skim milk powder was used as stabilizer at a dose of 2.5 g per liter of water; this was left for 60 minutes in the drinkers. The Newcastle vaccine used was La Sota strain, which was administered ophthalmically at 8, 20 and 35 days of age of the birds; the samples for counting antibodies to the same virus were taken on days 9, 21, 28, 35 and 42, at treatment V and VP. Weekly feed rejections were weighed on an OHAUS® model T21P scale with a precision of 50 grams and the average feed consumption was obtained. Feed conversion was obtained by the ratio of feed consumed per pen to the average weight gain per pen.

To measure Newcastle antibodies, on day 9, 0.3 mL of blood was taken from the jugular vein of 6 birds of each treatment; on days 21, 28, 35 and 42, 1 mL of blood was extracted from 24 birds of each treatment; sera were obtained and hemagglutination inhibition was performed to quantify antibody titers using the technique performed by González (2012).
A completely randomized statistical design was used using the following model:

$$y = \mu + Vi + Rj + \epsilon$$

Where:
y = the variable to be measured.
μ = the overall mean.
Vi = the ith level of polysaccharide utilization and vaccination.
Rj = the jth repetition effect.
ϵ = standard error.

The response variables were analyzed by ANDEVA and the comparison of means was analyzed by Fisher's method, both with a significance level of 5%. The Minitab 18 Copyright 2017® program was used to analyze the data; mortality was also analyzed using the chi-square method.

## RESULTS AND DISCUSSION

### Average feed consumption

In the second week, average feed consumption was significantly higher for the PC treatment, which consumed 28 and 36 g more than the V and C treatments, respectively ($P < 0.05$).

From week 4 to week 6 the feed consumption of the treatments was similar (Table 1). In a study conducted by Alaeldein *et al*., (2013), where diets were supplemented with *Ulva lactuca* algae, they found no difference between feed intakes; similarly there was no difference according to what was reported by Chávez *et al*., (2016). Evans *et al*. (2015) found that with a 21 % inclusion of spirulina algae, feed intake was significantly lower.

**Table 1. Weekly consumption per bird in grams**

| TREATMENT | | C | V | VP | PC | S.E. | P Value |
|---|---|---|---|---|---|---|---|
| WEEK | 2 | 387 ± 18 $^{c}$ | 395 ± 9 $^{bc}$ | 412 ± 20 $^{ab}$ | 423 ± 16 $^{a}$ | 5.42 | 0.005 |
| | 3 | 623 ± 14 | 620 ± 34 | 620 ± 34 | 634 ± 33 | 9.99 | 0.830 |
| | 4 | 874 ± 17 | 886 ± 44 | 879 ± 47 | 884 ± 36 | 12.65 | 0.953 |
| | 5 | 1087 ± 27 | 1103 ± 47 | 1097 ± 53 | 1092 ± 46 | 14.75 | 0.927 |
| | 6 | 1074 ± 32 | 1105 ± 58 | 1070 ± 59 | 1088 ± 64 | 18.2 | 0.696 |

$^{a,b}$ Different literals per row indicate statistically significant difference (P < 0.05).

## Weekly weight gain

In the first week of the experiment, it was observed that the treatments that were added with SP, the VP and the PC, obtained greater weight gain (P < 0.05). During week 2, PC treatment gained 16 to 29 g more compared to the other three treatments; the same occurred in week 4, where the differences were 17 to 42 g more; this difference was significant (P < 0.05). In the last week, no differences between treatments were observed; the weights are shown in Table 2. In a study conducted by Rezvani *et al*., (2012), where they supplemented diets with different percentages of inclusion of *Chlorella* algae and prebiotics, they did not obtain significant differences between their treatments; however, the weights from week 3 were higher than those of the present study, with the treatment with 0.07 % inclusion of *Chlorella* algae being higher.

Results obtained by Mariey *et al*. (2012) Mariey *et al.* (2012), in their study carried out with different percentages of spirulina algae inclusion, show a significant difference only with the highest percentage of this algae inclusion, which was 0.20 %, unlike the present study where only in week 4, two of the treatments with algae were significantly higher than treatments without algae. Evans *et al*. (2015), reported that treatments added with spirulina algae did not obtain a significant difference compared to the treatment without this algae; results similar to those obtained in the present study. In another study where *Ulva lactuca* algae was included in the diet, no significant differences were obtained compared to diets without algae (Alaeldein *et al.,* 2013).

**Table 2. Weekly weight gain (g) per bird**

| Treatment | Week | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| **C** | 171 ± 15 [b] | 420 ± 43 [b] | 751 ± 66 [a] | 1176 ± 137 [b] | 1704 ± 175 [ab] | 2205 ± 231 |
| **PC** | 174 ± 14 [a] | 436 ± 33 [a] | 741 ± 59 [ab] | 1205 ± 109 [a] | 1738 ± 168 [a] | 2218 ± 228 |
| **V** | 167 ± 14 [c] | 397 ± 32 [c] | 723 ± 65 [c] | 1163 ± 102 [b] | 1700 ± 160 [b] | 2209 ± 260 |
| **VP** | 177 ± 14 [a] | 413 ± 30 [b] | 728 ± 65 [bc] | 1187 ± 103 [ab] | 1712 ± 164 [ab] | 2177 ± 231 |
| **E.S.** | 0.002 | 0.005 | 0.010 | 0.018 | 0.027 | 0.0.039 |
| **P** | 0.000 | 0.000 | 0.000 | 0.011 | 0.195 | 0.473 |

[a,b]Different literals per column indicate statistically significant difference (P < 0.05).

## Feed conversion

Results of feed conversion are shown in Table 3, where it is observed that the PC treatment obtained a better performance compared to the V and VP treatments (P < 0.05). In a study conducted by Rezvani *et al.,* (2012), they obtained that feed conversion at day 42 of life was better in the treatments to which *Chlorella* algae was added in its different percentages of inclusion and in the treatment to which prebiotics were added compared to the control treatment. Evans *et al*. (2015), in their experiment where different inclusion percentages of *Spirulina* algae were tested, did not obtain a significant difference (P > 0.05), compared to the control diet. Alaeldein *et al.,* (2013) found no significant difference (P > 0.05) between the control treatment and those added with different inclusion percentages of *Ulva Lactuca*.

**Table 3. Average Feed Conversion by Treatment**

| Treatment | | | | | |
|---|---|---|---|---|---|
| **VP** | **C** | **PC** | **V** | **S.E.** | **P** |
| 1.8895 ± 0.19 [a] | 1.8707 ± 0.21 [ab] | 1.8334 ± 0.23 [b] | 1.8879 ± 0.25 [a] | 0.03 | 0.047 |

[a,b] Literales diferentes por fila indican diferencia estadísticamente significativa (P < 0.05)

## Hemagglutination Inhibition

The measurement of antibodies against Newcastle showed that at 9 days of sampling there was no difference between any of the treatments; at days 21, 28, 35, and 42, groups V and VP created more antibodies; this difference was significant (P < 0.05). Results show a higher production of antibodies in the groups to which vaccine was applied. In a study by Sedeik *et al.,* (2019), where they evaluated the generation of antibodies against Newcastle virus, comparing treatments without vaccine and with vaccines of different brands, they obtained that the antibody titers in week 2 and 3 post-vaccination, vaccinated groups were significantly ($P < 0.05$) higher than the non-vaccinated ones. Xu *et al.,* (2013), evaluated the multiplication of Newcastle virus in spleen, kidney, liver, lung and heart in two treatments; birds inoculated with the virus and birds inoculated with the virus and treated with sulfated polysaccharides, where they obtained that the virus titers in the spleen, heart and lung of the groups treated with polysaccharides were statistically lower ($P < 0.05$).

**Table 4. Antibodies against Newcastle disease**

| **Treatment** | **Days** | | | | |
|---|---|---|---|---|---|
| | **9** | **21** | **28** | **35** | **42** |
| **C** | 5.83 ± 1.84 | 3.83 ± 1.24 $^{b}$ | 2.5 ± 1.75 $^{c}$ | 2.80 ± 2.69 $^{c}$ | 7.38 ± 2.06 $^{b}$ |
| **PC** | 6.33 ± 1.21 | 4.08 ± 1.32 $^{b}$ | 4 ± 2.7 $^{b}$ | 4.30 ± 3.37 $^{b}$ | 6.58 ± 2.60 $^{b}$ |
| **V** | 7 ± 1.41 | 7.5 ± 1.62 $^{a}$ | 8.13 ± 1.15 $^{a}$ | 9.04 ± 1.68 $^{a}$ | 9.5 ± 1.06 $^{a}$ |
| **VP** | 6.5 ± 1.64 | 7.08 ± 1.14 $^{a}$ | 7.91 ± 1.32 $^{a}$ | 8.25 ± 1.85 $^{a}$ | 9.58 ± 1.25 $^{a}$ |
| **S.E.** | 0.515 | 0.223 | 0.305 | 0.415 | 0.309 |
| **P Value** | 0.633 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Coef. Var.** | 23.40 | 38.02 | 54.08 | 59.05 | 27.23 |

$^{a,b}$Different literals per row indicate statistically significant difference ($P < 0.05$). Values are expressed on base 2 corresponding to the hemagglutination inhibition titer value.

## Percentage of mortality

When analyzing mortality, it was found that the VP group obtained the highest percentage, being this difference significant compared to the PC group ($P < 0.05$). The percentages are similar to those reported by Gutiérrez *et al.,* (2015), who obtained a mortality percentage of 1.66 % in the control group; unlike the group supplemented with probiotics, which obtained a null mortality percentage. Similarly, the results coincide with the study conducted by Francia *et al.,* (2009), where the results ranged from 0.28 to 6.66 % mortality in the comparison of two genetic lines.

**Table 5. Mortality percentage**

| **Treatment** | **% Mortality** |
|---|---|
| C | 2.08%$^{ab}$ |
| V | 2.14%$^{ab}$ |
| VP | 3.59%$^{a}$ |
| PC | 0.00%$^{b}$ |

$^{a,b}$ Different literals per row indicate statistically significant difference ($P < 0.05$).

## CONCLUSION

The production of antibodies against Newcastle virus was shown to be higher in the groups in which the vaccine was administered; however, polysaccharides showed no effect on the response to the vaccine. Sulfated polysaccharides showed no effect on feed intake, weight gain and feed conversion.

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