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Utilization of mannan oligosaccharides as antibiotic substitutes in laying hens

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Abstract

Among global concerns about antibiotic resistance, it is necessary to identify food-safe alternatives to enhance production. This study aimed to evaluate the impact of mannan oligosaccharides (MOS) inclusion to replace antibiotic growth promoters (AGP) in the diets of ISA Brown laying hens aged between 23 and 31 weeks. Two hundred forty hens were grouped into five treatments: Control, AGP (130 ppm of enramycin 8.00%), and 100, 200, and 400 ppm of MOS. Each treatment had 16 experimental units (each unit with n = 3) in a randomized block experimental design. Productive data (egg production %, feed intake, egg weight), egg quality variables (albumin height, yolk weight, albumin, yolk %, weight eggshell, eggshell %, equator thickness, width-pole thickness, and Haugh units), organ weights (ovary, liver, and cecum) and jejunal histomorphometry were analyzed. The egg production was not affected by the substitution of AGP by MOS. Furthermore, MOS supplementation resulted in significantly increased feed intake, larger egg weight, higher yolk weight and higher body and ovarium weight compared to the AGP group. Besides, MOS supplementation at 400 ppm demonstrated significant improvements in jejunal villus morphology indicating enhanced intestinal health. These findings highlighted the potential of MOS as an alternative to AGP, offering benefits such as improved feed intake, egg quality and intestinal health in laying hens at 400 ppm.

Introduction

For over 80 years, antibiotics have been incorporated into animal feed at sub-therapeutic doses to promote growth, enhance feed conversion efficiency and prevent diseases.[1,2] Antibiotic growth promoters (AGP) improve digestibility and feed conversion, hence, promoting intestinal health.[3,4] However, the AGP used in animal nutrition is related to the appearance of antibiotic resistance.[5-7] There is a global concern to find alternatives to enhance production and identify safe alternatives to antibiotics for food safety.[8-10]

Probiotics, prebiotics and phytobiotics show promise as immunostimulants in animal nutrition.[11-13] Their effectiveness depends on factors such as animal species, dosage and administration timing.[14,15] These additives focus on preventing pathogenic infections and improving animal health.[16,17] They positively impact intestinal morphology and modulate the host immune response against gastrointestinal infections.[18,19]

Mannan oligosaccharides (MOS) are derived from the external cell wall of yeast such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii.*[20,21] They possess immunomodulatory characteristics[22,23] and have the ability to shift the balance of gut microbiota towards beneficial organisms.[24,25] In the small intestine, MOS, which remains undigested by the stomach continues to function and promotes the activity of beneficial intestinal microbiota.[26,27] The MOS act as intestinal microbiome modulators, playing a crucial role in activating defense mechanisms, enhancing the activity of digestive enzymes, improving nutrient utilization from feed, preventing the growth of pathogenic microorganisms and neutralizing pathogen-secreted toxins.[28,29]

The objective of this study was to assess the impact of supplementing ISA Brown laying hens in the peak production phase (Weeks 23 to 32) with three levels of MOS and without AGP on productive performance, egg quality and intestinal morphometry.

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Materials and Methods

Study location and animal care. This study was conducted in the experimental farm of the Universidad Cooperativa de Colombia in Ibagué at an altitude of 1,045 m above sea level (4°25'59''N, 75°13'1''W) with a thermal range of 20.70 and 30.60 ˚C, 92.00% relative humidity and 12 hr of daylight.

Animals and diets. The procedures were approved by the Bioethics Committee of the UDCA University, No. 03 122019. The ISA Brown laying hens were placed in individual cages. Eighty experimental units were utilized comprising a total of 240 ISA Brown laying hens from 23 to 32 weeks of age. The hens were housed in individual cages with one experimental unit assigned to every three consecutive cages. The animals were fed on the same base diet (Table 1) and water was offered *ad libitum*. The base diet was formulated according to National Research Council requirements.[30] At the beginning of the experimental period, intestinal dysbiosis was induced by supplying 10 times the normal dose of Fortegra MSD® vaccine (Intervet, Roseland, USA) orally, containing oocysts of *Eimeria acervulina, E. maxima, E. maxima MFP, E. mivate* and *E. tenella*.

Table 1. Chemical composition and perceptual composition of ingredients used in the experimental basal diet.

Ingredients	Percentage of dry matter
Yellow corn (8.50%)	53.60
Soybean meal (48.00%)	18.66
Wheat bran	8.51
Corn gluten meal	3.00
Palm oil	3.95
Di-calcium phosphate	1.82
Limestone, fine	4,49
Limestone, coarse	4.00
Vit-min premix*	0.20
DL-methionine	0.18
L-Lysine HCl	0.15
Salt	0.25
Sodium bicarbonate	0.10
Choline chloride 60.00%	0.005
L-Threonine	0.003
Zeolite	1.04
Chemical composition	
Crude protein (%)	17.02
Metabolizable energy(Kcal kg^{-1})	2,750.0
Calcium (%)	3.90
Available phosphorus (%)	0.29
Lysine (%)	0.90
Methionine (%)	0.44

* Vitamins and mineral premix. Containing *per* kg: Vitamin (Vit) A 7,500 UI; Vit. D_3 2,000; Vit. E 10.00 UI; Vit. Vit. K_3 1.80 mg; B_1 1.50 mg; Vit. B_2 4.00 mg; Nicotinic acid 25.00 mg; Pantothenic acid 10.00 mg; Vit B_6 1.70 mg; Vit B_{12} 0.013 mg; Folic acid 0.50 mg; Biotin 0.05; Choline 220 mg; Copper 11.00 mg; Iron 55.00 mg; Iodine 1.10 mg; Manganese 77.00 mg; Selenium 0.33 mg; Zinc 72.00 mg.

Treatments and experimental periods. A 2-week adaptation period was introduced at the onset of the peak production phase followed by 9 weeks of the experimental period. The animals were assigned to one of five treatments: Control (without feed additives), AGP (130 ppm of enramycin; Anhui Apeloa Biotechnology Co., Dongzhi, China) and 100, 200, and 400 ppm of MOS (Tekzol, Bogota, Colombia). During sample collection, daily feed consumption and egg weight were recorded from 23 to 32 week.

Productivity performance. The individual weights of the hens were recorded at 21 weeks of age and subsequently at 23 and 32 weeks during the experimental period. The measurement of feed intake and egg collection was conducted from weeks 23 to 32. Eggs were gathered daily and weighed fortnightly and daily mortality rates were recorded.

Egg quality parameters. For the assessment of egg quality, five eggs from every experimental unit were individually collected, overall, 80 eggs were collected fortnightly. The eggshell thickness was determined by measuring equally spaced positions along the longitudinal and equatorial axes of the egg using a manual micrometre. To determine the height of the albumen, an average of three measurements was taken using a tripod micrometer positioned between the yolk and the outer edge of the albumen.[31] The collected eggs were weighed and then the shells and contents were separated for quality measure-ments. The eggshells, albumin and yolk were individually separated and placed in a drying oven at 40.00 ˚C for 24 hr. Haugh units (HU) were calculated using the formula:

$$HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$$

where, H is the mean height (mm) of the albumen and W is the weight (g) of the egg.[32]

Intestinal morphometry. After the experimental period, a randomized sampling of 80 hens, 16 hens for treatment were selected for sacrifice by cervical dislocation.[33] The body, liver, ovary and cecum weights were measured. Two sections (2.00 cm) from the jejunum in the posterior region of Meckel's diverticulum were collected. These sections were washed with saline solution and then fixed in 10.00 % buffered formalin with proper labelling to be subsequently processed.[34] Quantitative analysis of the samples was performed using computerized digital image processing with a microscope and Zen Software (Carl Zeiss, Jena, Germany). Measurements included the height and width of the microvilli from apex to base and from edge to epithelial edge, respectively. The depth of the crypt was measured from the luminal epithelium to the beginning of the muscle layer. A total number of 10 measurements were taken *per* slide, selecting microvilli with the best integrity for analysis.

Statistical analysis. The data were subjected to an analysis of variance (ANOVA) using SPSS Software (version 26.0; IBM Corp., Armonk, USA) for Windows.[35] The differences between groups were determined by Duncan's multiple-range tests. All values were presented as means and standard errors of the mean, and significance levels were set as $p < 0.05$.

Results

There were no significant differences in egg production percentage, egg and yolk weight, and yolk percentage between the MOS supplemented diets and those containing AGP (Table 2). The addition of MOS at different levels (100, 200, and 400 ppm) showed increased ($p < 0.05$) feed intake compared to the AGP. Diets with MOS, especially at higher concentrations (400 ppm), resulted in increased egg weights ($p < 0.05$) compared to both the control and AGP.

The MOS supplementation did not significantly impact albumin height, albumin percentage, eggshell weight, eggshell percentage, eggshell equator thickness or haugh units among the treatments ($p > 0.05$)

In the body and organs weight (Table 3), the supplementation of MOS at 100 ppm and 400 ppm showed higher body and ovary weights ($p < 0.05$) compared to the Control and AGP group. The liver weights in the control group were higher ($p < 0.00$). There were no differences ($p = 0.53$) in cecum weights among the dietary groups.

In the jejunal villus morphology (Table 4), the results showed that MOS at 400 ppm increased villus height ($p < 0.05$). In the villus width, MOS at 100 ppm was similar to the AGP group ($p < 0.05$; Fig. 1).

Discussion

The MOS could replace AGP in the diets of laying hens increasing egg production, feed intake, egg weigh without affecting egg production. Similar findings were reported in Hy-Line Brown hens supplemented with MOS at various levels (0, 0.50, 1.00, 1.50, and 2.00 g kg^{-1} of diet). They observed that at levels of 1.00 and 1.50 g kg^{-1}, there was an increase in egg production percentage. Also, MOS supplementation improved the digestibility coefficients of crude protein and dry matter, while reducing ileal enumerations of *Escherichia coli* and total bacteria.[28]

Similarly, it was found that supplementation with MOS (1.00 g kg^{-1}) during forced molting resulted in improved egg production, egg weight and feed conversion ratio.[36] In the present study, hens fed on MOS showed increased feed

Table 2. Effects of mannan oligosaccharides (MOS) supplementation on productivity and qualitative parameters of eggs from 23 to 32 weeks.

Parameters	Control	AGP*	MOS inclusion (ppm)			Standard error of means	*p*-value
			100	200	400		
Productivity parameters							
Egg production (%)	70.91[b]	80.22[a]	79.93[a]	79.80[a]	79.61[a]	3.61	0.00
Feed intake (g *per* day)	92.80[c]	99.84[b]	102.12[a]	101.53[a]	101.94[a]	1.51	0.00
Qualitative parameters							
Eggshell equator thickness (µm)	429.30	405.92	418.43	417.75	423.52	6.70	0.14
Eggshell pole thickness (µm)	390.11	390.86	379.94	380.14	380.91	4.84	0.07
Albumin height (mm)	9.42	9.83	9.52	9.55	9.94	0.23	0.74
Egg weight (g)	56.33[c]	57.21[ab]	58.52[ab]	57.85[ab]	59.16[a]	4.19	0.00
Weight eggshell, (g)	5.91	5.56	5.87	5.60	5.93	0.09	0.05
Yolk weight (g)	12.86[b]	13.37[ab]	13.67[a]	13.14[a]	13.23[a]	0.23	0.00
Eggshell (%)	10.45	9.91	10.26	10.21	10.15	0.14	0.08
Albumin (%)	67.41	66.10	661.01	66.53	67.46	0.35	0.14
Yolk (%)	22.48[b]	24.03[a]	23.72[a]	23.43[a]	23.54[a]	0.33	0.00
Haugh units	97.01	97.32	97.36	97.92	98.62	0.87	0.65

Data in ISA Brown laying hens from 23 to 32 weeks.
* AGP: 130 ppm of antibiotic growth promoter enramycin 8.00 %.
[ab] Means with different letters have statistical differences by Duncan test.

Table 3. Effects of mannan oligosaccharides (MOS) supplementation on body and organ weight from 23 to 32 weeks

Parameters	Control	AGP*	MOS inclusion (ppm)			Standard error of means	*p*-value
			100	200	400		
Body	1,570.58[b]	1,486.43[b]	1,640.12[a]	1,562.20[b]	1,642.61[a]	19.28	0.00
Ovarium	26.80[c]	26.80[c]	38.40[a]	33.13[b]	36.58[ab]	0.93	0.00
Liver	46.65[a]	33.60[b]	36.80[b]	35.22[b]	36.50[b]	1.13	0.00
Cecum	9.50	8.62	10.20	9.92	9.82	0.37	0.53

Data in ISA Brown laying hens from 23 to 32 weeks.
* AGP: 50.00 ppm of antibiotic growth promoter enramycin 8.00%.
[abc] Means with different letters have statistical differences by Duncan test.

Table 4. Effects of mannan oligosaccharides (MOS) supplementation on jejunal villus morphology from 23 to 32 weeks.

Parameters	Control	AGP*	MOS inclusion (ppm)			Standard error of means	*p*-value
			100	200	400		
Villus height (µm)	752.90^{bc}	772.80^{b}	723.28^{b}	723.28^{c}	822.28^{a}	21.77	0.00
Villus width (µm)	165.70^{b}	168.98^{b}	155.70^{b}	146.19^{a}	147.5^{a}	4.09	0.00
Crypt depth (µm)	126.90^{c}	145.50^{a}	138.20^{b}	136.04^{b}	128.44^{c}	3.26	0.00
Villus height : crypt depth ratio	6.16^{a}	5.39^{b}	5.66^{b}	5.32^{b}	6.53^{a}	0.81	0.00

Data in ISA Brown laying hens from 23 to 32 weeks. * AGP: 150 ppm of antibiotic growth promoter enramycin 8.00%.
^{abc} Means with different letters have statistical differences by Duncan test.

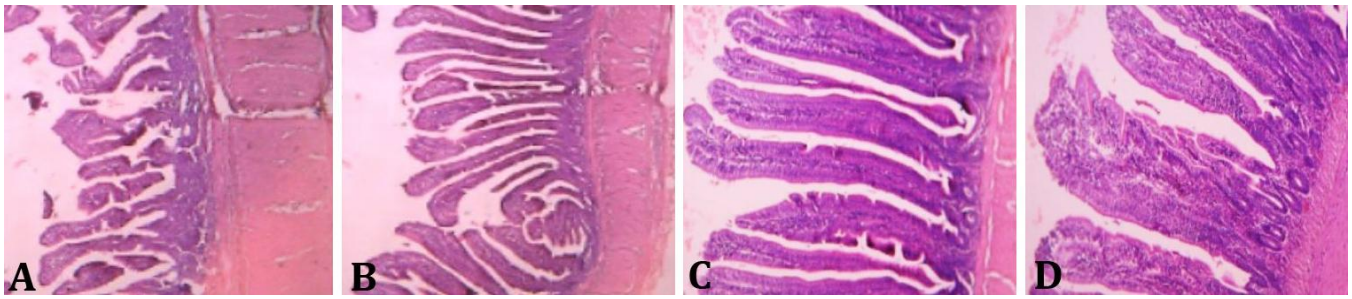

Fig. 1. Histological micrographs of the small intestinal villus under different treatments using Hematoxylin and Eosin staining. **A)** Control: standard villus structure without supplementation (4×), **B)** Mannan oligosaccharides (MOS) 400 ppm: higher villus height and improved integrity compared to the control (4×), **C)** MOS 400 ppm: villus height can be observed (10×), and **D)** MOS 100 ppm: villus width (10×). The control group had a deeper crypt compared ($p < 0.00$) to the AGP and MOS groups. The lowest crypt depth was observed in the MOS 400 ppm group. The MOS at 400 ppm resulted in a higher ratio villus height : crypt depth ratio ($p < 0.05$).

intake and higher MOS concentrations (400 ppm) positively affected egg size. The addition of MOS at various levels did not compromise egg quality characteristics in comparison with AGP group. This underscored the potential of MOS to maintain egg quality. Correspondingly, these results were validated in Japanese quails supplemented with 0.25, 0.50 and 1.00% of MOS, respectively, without impacting the quality parameters or egg geometry.[37]

No significant changes were observed in shell weight, percentage or thickness at the measurement points contrasting with the effects of 2.00 g *per* ton of MOS in 32-week-old hens.[38] On the other hand, supplementation with 1.00 g kg^{-1} of MOS positively affected eggshell quality in 60-week-old reproductive females,[39] yet had no impact on post-molting hens. Though, at 70 weeks of age, concentrations of 0.40 and 0.80% resulted in a significant increase in albumin height, production percentage and eggshell thickness.[40] These discrepancies may arise from direct and indirect mechanisms exerted by MOS in response to physiological changes associated with aging birds particularly in calcium and phosphorus metabolism. The present findings were primarily attributed to the increased digestibility of ileal nutrients and reduction of pathogenic gut bacteria.

Supplementation with MOS, at 400 ppm, positively influenced the overall body weight as well as the weight of the ovary. This suggested a beneficial impact on ovarian development potentially enhancing reproductive performance in laying hens. On the intestinal morphology, higher concentrations of MOS (400 ppm) had a positive influence on the height of the small intestinal villi potentially enhancing nutrient absorption.

The MOS at low levels (100 ppm) showed the same effect that AGP group in the villus width, which could be relevant to the absorptive surface area. Shallower crypts were observed at high levels of MOS (400 ppm) reflecting the prolonged survival of villi without the need for renewal with reduced energy expenditure for this process and the consequent growth of other tissues.[41] The ratio of villus height to crypt depth is an important indicator of intestinal health. This suggests that the higher concentration of MOS (400 ppm) promoted a favorable balance between villus height and crypt depth.

A greater height of intestinal villi is associated with reduced inflammatory processes in the intestinal mucosa and an increase in local innate immunity,[42,43] which in turn enhances the secretion, digestion and absorption of nutrients due to prolonged and closer contact between intestinal contents and the absorption surface.

According to the findings of our study, supplementation with 400 ppm of MOS increased villi height by 9.40% and decreased villi width by 11.00% compared to the AGP. Increased height and width of duodenal villi were observed in quails supplemented with 2.00 g kg^{-1} of MOS.[44,45] Furthermore, studies on broilers supplemented with MOS showed increased height of jejunal villi with no effect on crypt depth.[46]

Our results show that MOS at 400 ppm could positively influence egg weight and overall egg quality without compromising egg production or feed intake, and had a positive influence on the height of the small intestinal villi, potentially enhancing nutrient absorption. These findings indicated the potential of MOS as a viable alternative to AGP in laying hen nutrition.

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Conflict of interest

The authors declare no conflicts of interest. The funders had no role in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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