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Effects of NSPase Supplementation on Growth Performances, Histological and Macroscopic Lesions and Coccidiosis Load of Broiler Chickens

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Abstract

The development and use of enzymes has gained momentum in poultry nutrition as an alternative to growth-promoter antibiotics use. Non-starch polysaccharide degrading enzymes (NSPase) prevent the accentuation of viscosity in the gastrointestinal tract and its associated negative effects, which will allow better digestion and absorption of nutrients, reduced microbial proliferation and healthier gut. The objective of this study is to evaluate the effects of several NSPases on growth performances, intestinal histomorphology, macroscopic lesions and coccidiosis loads of broiler chickens. A total of 2400 one-day-old male chicks (Ross 308 strain) were weighted and randomly assigned to 80 floor pens at the rate of 30 birds/pen. A total of 10 treatments have been tested with 8 replicates each. Eight groups received basal diets supplemented with β-xylanase, β-glucanase, α-amylase, xylanase, cellulase, mannanase and α-galactosidase at different rates. Two control diets were formulated to meet the nutrient requirements: 1) positive control (PC) (diet with Avilamycine) and negative control (NC) (diet, without antibiotic and NSPases). The main results showed that supplementation of 50 g of β-xylanase and β-glucanase mixture (T3) induced significant ($p < 0.05$) body weight (BW) and body weight gain (BWG) increases and a very highly ($p < 0.001$) significant decrease in feed conversion ratio (FCR) during the period of 14 to 21 d of age. Moreover, T3 had very highly ($p < 0.0001$) significant effects on coccidiosis evolution from D14 to D26, as chicks get coccidiosis in cæcum only at day 26 with a load score of "1". In addition, the lowest ($p < 0.01$) macroscopic intestinal lesion score was observed in T3 group at day 14 (S = 0,75). For histological analysis, T2 (50g of β-xylanase and β-glucanase mix/ton) and T6 (100 g of a mixture of xylanase, cellulase, β–glucanase, mannanase, α-galactosidase/ton) presented the lowest ($p < 0.001$) total ISI score. Therefore, we conclude that supplementation of 50 g of a mixture of β-xylanase and β-glucanase per ton of feed improves growth performances, reduces coccidiosis loads and decreases macroscopic and microscopic lesions in broiler chickens.

Keywords: *NSPase; GPA; Broiler; Growth Performance; Lesion; Coccidiosis*

Introduction

To meet the increasing human demand for white meat, the use of new farming techniques to intensify production has been imposed. The most commonly known is the use of growth-promoter antibiotics (GPA) in broiler farms [1]. Thus, the rapid emergence of antibiotic resistance in human and animal health might be linked to the use of GPAs in poultry farming. Previous studies have established a direct relationship between the excessive and uncontrolled use of antibiotics in poultry farming and the antibiotic resistance reported in humans [2]. Therefore, thinking of GPAs use in chicken feed is highly recommended to come up with cofactors, of natural origins, to substitute GPAs. Indeed, several alternatives to GPAs have been identified such as enzymes, out of which the most widely used are the so-called non-starch polysaccharide degrading enzymes (NSPases).

According to the literature, NSPases are proteins with catalytic properties that metabolize non-starch polysaccharides (NSP) mainly in viscous cereals (wheat, barley and triticale) into oligosaccharides millions of times faster than in their absence [3-5]. Thus, this degradation allows poultry to metabolize cereal diets, based on an oligosaccharide and not on a non-starch polysaccharide [6,7]. As a result, the development and use of enzymes has gained momentum in poultry nutrition. NSPases prevent the accentuation of viscosity in the gastrointestinal tract and its associated negative effects [8-10], which will allow better digestion and absorption of nutrients, reduced microbial proliferation and healthier gut [11-13]. Indeed, when xylanase is used, for example, with rye, triticale, and wheat-based diets and gluconate with barley-based diets, the positive effects of enzyme supplementation are mainly due to the reduction of the viscosity of the digest and the partial hydrolysis of the soluble NSP fraction [14,15]. In addition, the administration of β-mannanaseand NSPases (carbohydrate cocktail: xylanase, β-glucanase and α-galactosidase) at 363.2 g/t of feed (159500VU/g of product) and 113.5 g/t feed (2700 VU/g product), respectively, in an energy-reduced diet (88 or 132 kcal/kg of apparent metabolizable energy (AME) depending on the growth stage) improves zootechnical performances and reduces broiler mortality to levels similar to those of the positive control (PC) [16].

Indeed, broilers supplemented with xylanase host a negligible number of *C. perfringens* as compared to control individuals [17] as well as reduced numbers of coliforms and *Salmonella* in the ileum [18]. Dietary supplementation with xylanase in broilers challenged with *Salmonella* spp. effectively induced a 61% reduction in *Salmonella* in positive cæcal samples compared to the control group [19].

The objective of this study is to evaluate the effects of different NSPases with different concentrations on growth performances, intestinal histomorphology, macroscopic lesions and coccidiosis loads of broiler chickens.

Materials and Methods

Animals and housing

The animal experiment took place from February the 24th till April the 6th, 2020 in environmentally controlled poultry house with static ventilation. A total of 2400one-day-old male chicks (Ross 308 strain) were weighted and randomly assigned to 80 floor pens of 3 m^2 (1,5 m × 2 m) at the rate of 30 birds/pen therefore each pen provided 0.1 m^2/bird with high quality straw litter. Temperature and relative humidity were maintained within an optimal range. Water and feed were provided ad libitum by a drinker and a feeder in each pen.

Experimental design

The chicks were allotted as per randomized block design containing ten groups (treatments) with eight replicates each and 30 birds per pen to end with 240 birds/treatment. Basal diet was formulated to meet nutrient requirements according to the Ross 308 manual. All diets were freshly prepared in mash form every week. Eight groups received basal diets supplemented with β-xylanase, β–glucanase, α-amylase, xylanase, cellulase, mannanaseand α -galactosidase at different concentrations and compositions. Two control diets were formulated to meet the requirements of nutrients:1) positive control diet (PC, with Avilamycine) and negative control diet (NC, withoutantibiotic and NSPases) (Table 1).

Treatment	Identification	Viscosity (VU*/g)	Doses (g / ton of feed)
T1	Endo 1,4 β-xylanase Endo 1,4 β–glucanases Endo 1,3 (4) β glucanases	-	100
T2	Endo-1,4-β-xylanase Endo-1,3(4)-β-glucanase	22 000 30 000	50
T3	Endo-1,4-β-xylanase Endo-1,3(4)-β-glucanase	25 000 17 200	50
T4	Endo-1,4-β-xylanase α-amylase β–glucanases	-	200
T5	Endo-xylanase α-amylase	-	100
T6	Xylanase Cellulase β–glucanase Mananase α -galactosidase	-	100
T7	1,4- β-xylanase	-	100
T8	1,4- β -xylanase Endo 1,4- β -glucanase	-	100
NC	No enzymes and no antibiotic	-	-
PC	Avilamycine	-	10

Table 1: *Composition of the different dietary treatments.*

: Viscometrical Unit.

Samples' collection

On days 14 and 26, five birds per treatment were randomly taken for samplings of internal organs and contents. Birds were humanly sacrificed by dislocation of the cervical vertebrae and dissected individually. Liver's right lobe and 1.5 cm intestine sections from the midpoint of duodenum, ileum and caecum were collected from each bird and fixed in a 10% formaldehyde solution in a sterile manner for histological analysis. The whole cadaver lesions were checked according tothe Growth Promoter alternative Standard (GPaS) method.

Growth performances

Fifteen chicks per pen were randomly picked up and individually weighed at a weekly basis. Since the beginning of the study, feed intake (FI) and mortality were daily recorded. This data allowed calculating the major growth parameters, namely: live body weight (BW) and body weight gain (BWG), mortality rate (MR) and feed conversion ratio (FCR).

Coccidiosis analysis

Coccidiosis analysis was processed using the flotation technique. One gram of cæcal and ileal contents were diluted in salted solution and oocysts were observed on glass slides under an optical microscope (100 x).

Macroscopic lesions' examination

On days 14 and 26, macroscopic alterations were evaluated using I See Inside (ISI®) [20] methodology and Growth Promoter alternative Standard (GPaS) method based on a digital score of alteration with the evaluated parameter combined with an impact factor (Related to the impact of the alteration on the functional capacity). Attributed scores range from 0 to 3 where score 0: absence of lesions; score 1: alteration in up to 25%; score 2: alteration ranges from 25.1% to 50% and score 3: alteration extends to more than 50% of the areas or observed frequencies of the four scores. An impact factor (IF) is defined for each alteration in macroscopic analysis, according to the reduction of organ functional capacity, based on previous knowledge from the literature and background research.

Histological analysis

Samples were dehydrated, infiltrated and embedded in paraffin following common histological routine. Blocks were sliced in 5 μm sections and stained with hematoxylin and eosin [21]. An average of 20 intestinal villi per slide per bird were observed for evaluation in 10 x magnitude (using 40 x objective to confirm alterations) of an optical microscope. Liver samples were evaluated in 10 fields per bird in 10 x objective [21]. In this methodology, an impact factor (IF) is defined for each alteration in microscopic analysis, according to the reduction of organ functional capacity, based on previous knowledge from the literature (i.e., necrosis has the highest IF because the functional capacity of affected cells is completely lost). The IF ranges from 1 to 3, with 3 being the most impacting to organ function. For pathological features defining normal histology, attributed scores range from 0 to 3, with score 0: absence; score 1: alteration in up to 25%; score 2: alteration ranges from 25.1 to 50% and score 3: alteration extends to more than 50% of the areas or observed frequencies. For microscopic lesions, the used scoring system is as follows: 0: absence; score 1: alteration in up to 25%; score 2: alteration ranges from 25.1 to 50% and score 3: alteration extends to more than 50% of the areas or observed frequencies. To obtain the final value of the ISI index, the IF of each alteration is multiplied by the respective corresponding score number, and the results of all alterations are summed according to the formula $ISI = \Sigma(IF*S)$, where IF is the impact factor and S the Score.

Statistical analysis

The analysis of variance and the estimation of the effects of treatments on the studied variables i.e.: growth performances, intestinal histomorphology, macroscopic lesions and coccidiosis loads were done using the method of generalized least squares of the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) program [22].

The analysis of variance for all studied variables is carried out under a fixed model including the Treatment factor in the factorial component. The mathematical formula of the model is as follows:

$Y_{ij} = \mu + T_j + e_{ij}$, where:

Y_{ij}: Studied variable (growth performances, intestinal histomorphology, macroscopic lesions and coccidiosis loads) for observation i under treatment T_j;

μ: General average;

Ti: Fixed treatment effect;

e_{ij}: Random error relating to the i^{th} observation (broiler chicken).

The Probability of Difference (PDIFF) and Theoretical Difference (TDIFF) options of the GLM procedure were used to obtain the values of the two-by-two equality test statistics of the adjusted means and the associated critical probabilities.

Results

Growth performances

The present study investigated the potential efficiency of non-starch polysaccharide degrading enzymes (NSPases) to help mitigate the negative impacts of anti-nutritional properties of partially water-soluble NSP in broiler chickens raised without antibiotics. The effects of dietary NSPase supplementation on broiler chickens' performances are shown in table 2. In fact, the effect of treatment on production performances was significant during the period of 14 to 21 d of age. Indeed, we observed a very highly ($p < 0.001$) significant reduction in feed conversion ratio (FCR) in the treatment 3 (T3) group as compared to the PC group. FCR was also significantly ($p < 0.05$) decreased in T2, T6, T7 and T8 lots as compared to the NC group. The highest ($p < 0.05$) body weight (BW) was recorded in T3 treatment as compared to T5, T7, T8 and NC treatments. However, body weight gain (BWG) was significantly ($p < 0.001$) increased in T3 treatment as compared to T4, T5, T6, T7, T8, NC and PC.

	Age (d)	Treatments									
		1	2	3	4	5	6	7	8	NC	PC
BW (g)	7	137.19	142.55	142.26	141.82	139.74	142.22	132.42	141.76	134.52	140.60
	14	411.41	396.91	398.16	400.50	385.67	397.09	375.85	395.51	374.39	398.13
	21	747.87 ^{a}	771.72 ^{e}	816.33^{a,b,c,d,f}	771.81 ^{g}	748.87 ^{b}	777.60 ^{h}	741.00 ^{c}	767.19^{d,i}	712.90^{e,f,g,h,i,j}	768.66 ^{j}
	28	1285.55	1349.08	1302.77	1313.21	1307.37	1308.05	1270.66	1298.17	1226.84	1297.58
	35	1724.09	1797.0^{r}	1740.03	1698.10	1728.41	1689.11	1775.78	1724.92^{r}	1663.78	1778.24
BWG (g)	1-7	13.43	14.30	14.20	14.17	13.88	14.08	12.88	14.12	13.15	13.91
	8-14	39.17	36.33	36.55	36.95	35.13	38.78	34.77	36.25	34.26	36.79
	15-21	48.06	53.54	59.73	53.04	51.88	54.35	52.16	53.09	48.35	52.93
	22-28	76.81	82.48	69.49	77.34	79.78	75.77	75.66	75.85	73.42	75.56
	29-35	62.64	63.98	62.46	54.98	60.14	54.43	72.15	60.96	62.41	68.66
	1-35	49.43	51.60	49.91	48.69	49.58	48.39	50.98	49.47	47.68	51.03
FI (g)	1-7	104.26	88.38	103.84	105.27	95.02	219.01	94.01	104.9	103.58	109.66
	8-14	386.72	385	386.21	384.48	385.67	385.17	390.88	387.59	385.62	382.20
	15-21	979.70	949.21	979.59	987.91	966.04	987,55	941.07	958.98	941.02	1022.31
	22-28	1851.19	1915.69	1849,93	18337.09	1843.39	1844.35	1842.45	1856.38	1815.72	1829.58
	29-35	2689.58	2749.41	2714.44	2682.99	2696.31	2668.79	2681.42	2690.87	2711.96	2738.48
FCR	1-7	0.76	0.69	0.73	0.74	0.68	1.54	0.71	0.74	0.77	0.78
	8-14	0.94	0.97	0.97	0.96	1.00	0.97	1.04	0.98	1.03	0.96
	15-21	1.31	1.23	1.2	1.28	1.29	1.27	1.27	1.25	1.36	1.33
	22-28	1.44	1.37	1.42	1.40	1.41	1.41	1.45	1.43	1.48	1.41
	29-35	1.56	1.53	1.56	1.58	1.56	1.58	1.51	1.56	1.63	1.54

***Table 2**: Effects of different NSPases' supplementation on growth performances in broiler chickens.*

Means within the same line with different exponent do differ significantly.

BW: Body Weight, FI: Feed Intake, FCR: Feed Conversion Rate.

Coccidiosis analysis

As shown in Figure 1 (A and B), the evolution of coccidiosis loads in broiler chickens from 14 to 26 days of age indicated that treatments had very highly ($p < 0.0001$) significant effects on coccidiosis. Indeed, from day 14 to day 26, coccidiosis regressed in T2, T6, T8 and NC treatment groups from ileum and at T5 and PC treatments from cæcum. However, T3 treatment chicks get coccidiosis in cæcum only at day 26 with a load score of "1".

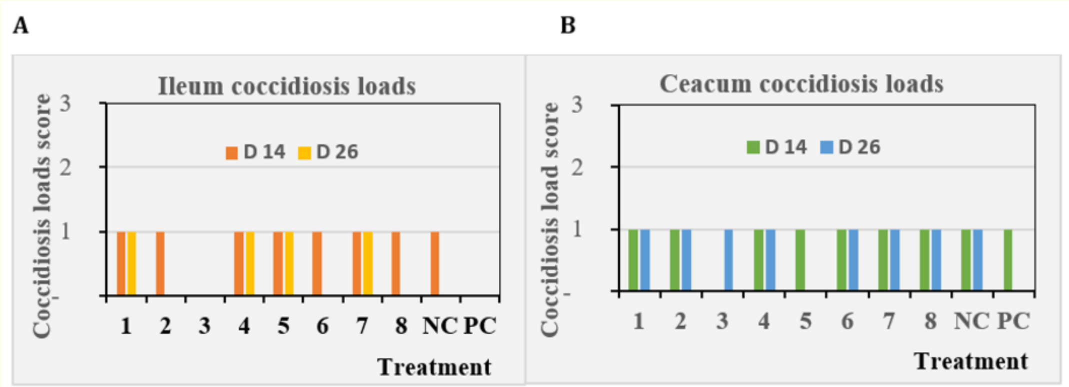
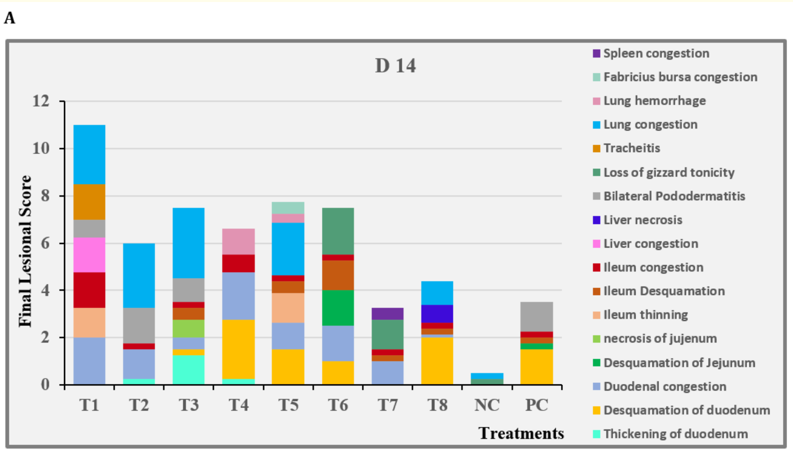


***Figure 1:** Effects of dietary supplementation of different NSPases (treatment) on ileum (A) and cæcum (B) coccidiosis loads at 14 (D14) and 26 (D26) days of age in broiler chickens.*

Macroscopic lesions

The results of the effects of dietary supplementation of the different NSPases are represented in figure 2 (A and B). At day 14, the T1 and T4 groups presented the highest ($p < 0.05$) macroscopic lesion scores in the respiratory system, as a consequence of tracheitis and lung congestion and in the intestinal tract ($p < 0.01$) due to duodenal desquamation and congestion, respectively. The T1 group showed also the highest ($p < 0.01$) liver lesion score as resulting from necrosis and congestion. However, the lowest ($p < 0.01$) macroscopic intestinal lesion score was observed in T3 group. At day 26, macroscopic lesions on different organs do not differ significantly ($p > 0.05$) among treatments except for lung congestion where T1 group showed higher ($p < 0.05$) lesion score.



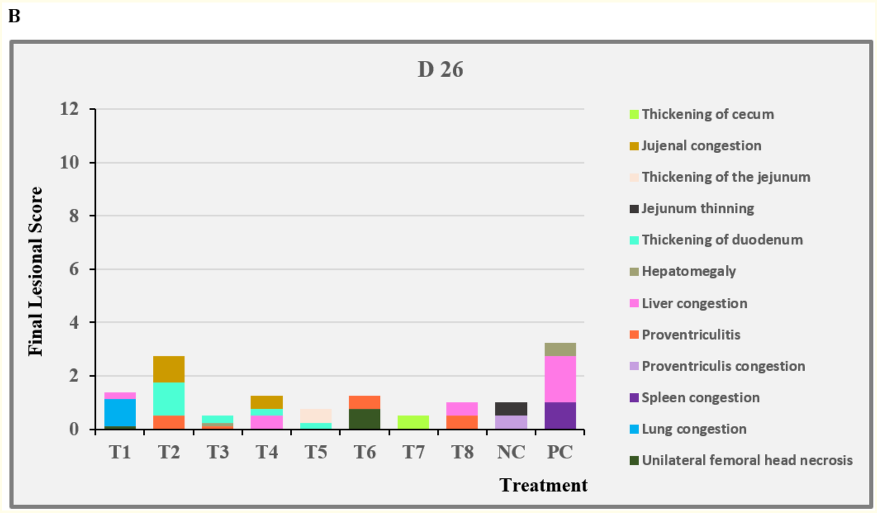
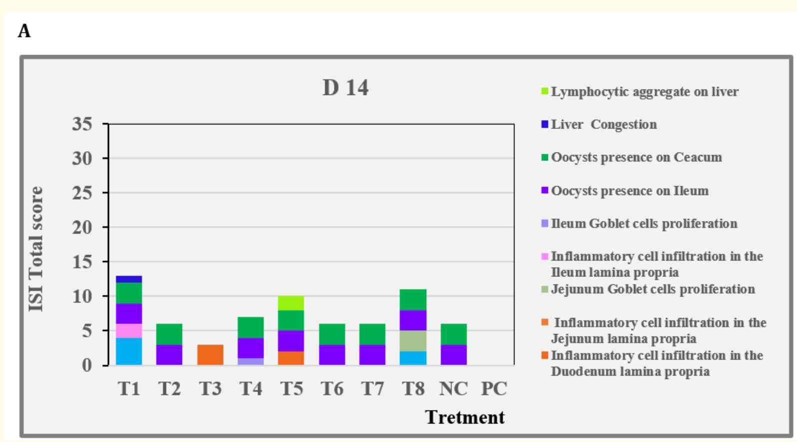


Figure 2: *Effects of dietary supplementation of different NSPases (treatment) on the final lesional score at 14 (D14) and 26 (D26) days of age in broiler chickens.*

Histological analysis

The effects of different NSPase supplementations were evaluated using histological analysis and results are shown in figure 3 (A and B). At day 14, histological lesions on different intestinal portions and liver were not significantly ($p > 0.05$) different among treatments. At day 26, several intestinal lesions are common to all intestinal portions whereas others are very highly ($p < 0.001$) different according to treatments. In fact, treatments T2 and T6 presented the lowest total ISI score while T1 and T7 presented the highest ISI total score as a consequence of coccidiosis (Photo 1. A) ($p < 0.001$). Histological evaluation in the liver showed that T1 group presented the highest ($p < 0.05$) ISI total score due to immune cell infiltration and congestion (Photo 1. C). Moreover, histological parameters in the cæcum showed an increase of lamina propria and epithelium thicknesses in T6 group relatively to the remaining treatments ($p < 0.001$).



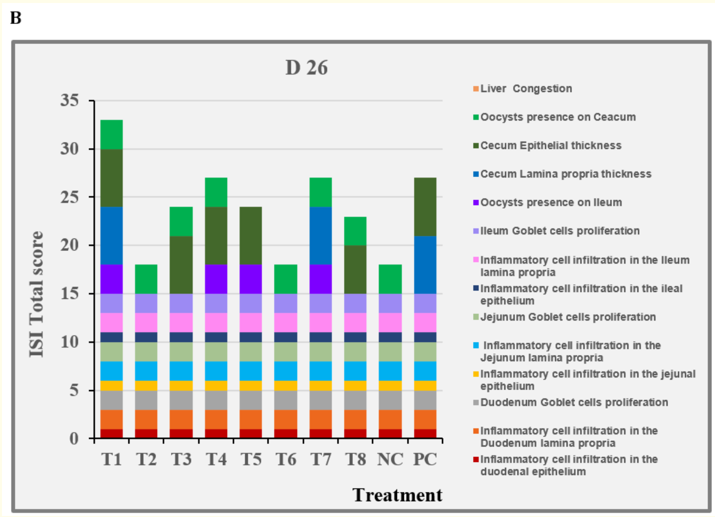


Figure 3: *Effects of dietary supplementation of different NSPases (treatment) on the total histological score at 14 (D14) and 26 (D26) days of age in broiler chickens.*

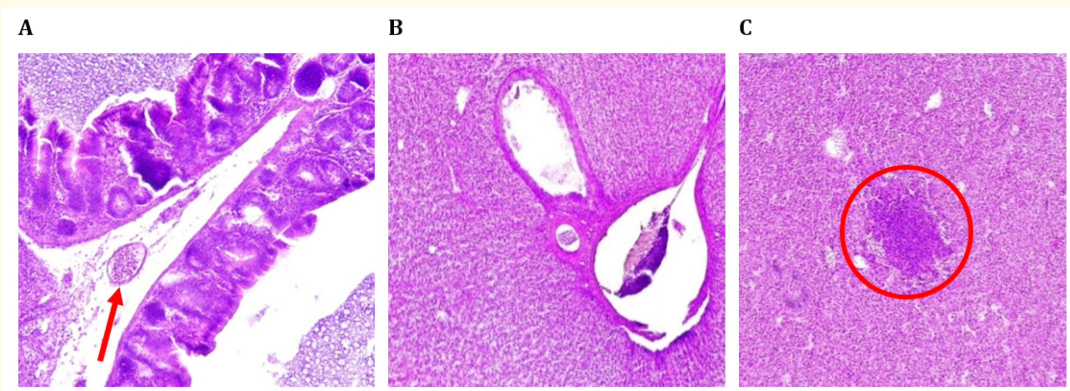


Photo 1: *Histological structures (100 x) of A: Cæcum with oocyst (arrow) in the T1 group, B: Normal liver in the T2 group and C: Inflammatory cell infiltration (circle) and congestion of the liver in the T1group; These alterations contributed to the highest ISI index (p< 0.001 on caecum and p< 0.05 on liver).*

Discussion

In the present study, we investigated the effects of different non-starch polysaccharide degrading enzymes (NSPases) on growth performances, macroscopic and histological lesions and coccidiosis load in broiler chickens. In an attempt to reduce the anti-nutritive effects of NSPs, exogenous enzyme supplementation in poultry has been shown to be effective, especially in high fiber diets. In the current study, supplementation of 50 g of a mixture of β-xylanase and β-glucanase (T3) per ton of feed reduced FCR and increased the BW and BWG at day 21 of age compared to other treatments including the PC. Indeed, the administration of xylanase, β-glucanase and α-galactosidase at 113.5 g/t of feed (2700 VU/g product) improves zootechnical performances and reduces broiler mortality to levels similar to those of the positive control (PC) [16]. Moreover, use of 0.01% of a mix of cellulase, glucanase, and xylanase in broilers significantly optimizes weight gain by 3.04% [23]. Xylanase supplementation also appears to improve the average daily gain in 20-25-day old broilers by 2.5 g/day and decreases the conversion index by 6 points compared to the negative control [24]. Indeed, NSPases' supplementation of 75 g/ton of feed with a cocktail of 203 IU of xylanase, 60 IU of cellulase and 53 IU of glucanase per kilogram of supplement improves the apparent ileal digestibility of polysaccharides and proteins by 2% and 23%, respectively [25] and consequently, the nutritional value of the grain [26]. Moreover, due to NSPases' ability to soften the "cage effect" of NSP [4,27], digestive viscosity was reduced with dietary supplementation with a mixture of 2500 VU of xylanase and 250IU of β-glucanase per kilogram of feed as well as in jejunal digest viscosity [9] which allows pancreatic enzymes to better digest nutrients and improve their absorption by intestinal villi [26].

On the other hand, coccidiosis has had major impacts on commercial poultry production by increasing mortality and decreasing performances of chickens in both clinical and sub-clinical forms [28-30]. In the present study, dietary intake of 50 g/ton of feed of β-xylanase and β-glucanase mix eliminated oocysts from the ileum in T2 group between D14 and D26 and prevented coccidiosis infestation of thecæcum portion in T3 group. Indeed, viscous grains may cause wet and sticky litter conditions, creating favorable conditions for bacterial and coccidian growths in the environment. As consequence, coccidia invade the intestinal epithelium causing damage to gut integrity and lesions, which reduce BWG and increase FCR. In our assay, broilers were naturally challenged with *Eimeria spp*. as obviously known, NSPases prevent the accentuation of viscosity in the gastrointestinal tract -and its associated negative effects- [8-10] which will allow better digestion and reduce microbial proliferation [11-13]. Indeed, when xylanase is used, for example, with rye, triticale, and wheat-based diets and glucanase with barley-based diets, the positive effect of enzyme supplementation is due, mainly, to the reduction of the viscosity of the digest and the partial hydrolysis of the soluble NSP fraction [14,15]. This experiment suggests that β-xylanase and β-glucanase mix may have beneficial effects in broilers during a coccidia infection by reducing digest viscosity which limits the risk of a humid litter conducive to coccidiosis, thus ending to break the life cycle of *Eimeria* spp.

The autopsies carried out at 14 d and 26 d allowed to highlight the evolution of the macroscopic lesions in each treatment. Actually, the lowest macroscopic intestinal lesion score noticed at 14 d was observed while chicks were daily supplemented with a mixture of 50 g/tone of feed of β-xylanase and β-glucanase (T3) (S = 0,75). However, at 26 d, macroscopic lesions on the different organs were not significantly ($p > 0.05$) different among treatments. Those results suggest that a cocktail of β-xylanase and β-glucanase could potentially decreases macroscopic lesions by reducing viscosity and speeding up the intestinal transit. Therefore, the more fluid the transit is, the less the intestinal barrier will be damaged. As previously reported, the combined supplementation of xylanase, protease and amylase in the diets of broilers vaccinated with live coccidia oocysts and challenged with mixed *Eimeria spp*. reduces the average intestinal lesion score compared to NC and PC [31].

Using ISI histological evaluation in intestines and liver, dietary supplementation of 50 g of β-xylanase and β-glucanase mix/tone of feed (T2) decreased total ISI score compared to all treatments at 26 d ($p < 0.001$). This may result from the effect of degrading enzymes on reducing digest viscosity and decreasing the "cage-effect" of NSP. This interaction could minimize the disposal of water-rich droppings and therefore a wet litter suitable for the sustainability of the *Eimeria* spp. life cycle. Indeed, the abundance of *Eimeria* oocysts depends on a number of factors including the type litter moisture content [32,33]. For example, the *E. maxima* oocyst sporulation in litter containing 5% moisture increases to 16% during 106h and in litter containing 60% moisture *E. maxima* oocyst sporulation increases to 62% [34].

Conclusion

Improvements on the availability of essential nutrients combined to dietary enzymes supplementation may provide protection against intestinal pathogens, activate the immune response, or enhance nutrient utilization to reduce the detrimental impact on growth performance and intestinal integrity. These data suggests that dietary supplementation of β-xylanase and β-glucanase cocktail may improve growth performances, reduce coccidiosis loads and could potentially be a cost saving strategy for producers. Understanding the interactions between NSPase mix in the broiler diets requires maintaining an adequate balance between substrate, nutrient content and enzyme matrix. However, in spite of performances, coccidiosis loads, macroscopic and histological lesions, non-starch polysaccharide degrading enzymes may not be used as a GPA alternative but rather considered as supplements to be incorporated in the poultry feed as for vitamins or organic acids.

Authors Contributions

DS and NS collected samples used in the study after an autopsy. DS performed all laboratory analysis, carried out the slide reading, analyzed obtained data and wrote the original draft. JA performed statistical analysis. NS and OM revised and edited the draft to generate the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Volume 7 Issue 5 May 2022