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An Algal Extract Improves Liver Function and Laying Performance of Turkey Breeders

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Abstract: The digestive system of poultry breeders is highly solicited, especially late in the egg production period when the demand in lipids for yolk development increases. To maintain a good health status and optimal levels of egg production, the digestive system needs to be properly drained and stimulated. This study assesses the impact of the product DigestSea® containing a sulphated polysaccharide extracted from green seaweeds named MSP®$_{LIPIDS}$, on liver physiology and laying performance of breeding turkeys in comparison with a choline and sorbitol complex. The trial was performed in two buildings, from 23 to 28 weeks of laying. One group of 2,016 turkey breeders received the choline and sorbitol complex at 1 L/1,000 L of drinking water while the test group of 2,110 turkey breeders received DigestSea® at a dosage of 0.5 L/1,000 L of drinking water, during 2 periods, first 10 days at 23 weeks of laying and again 5 days at 27 weeks of laying. Histological evaluation of the liver was performed at the end of the trial. The results show higher egg production in the DigestSea® group with a significant reduction of the biliary canal hyperplasia and blood capillaries leucocytosis in the portal area, associated with a statistically significant reduction of lipids overload in the liver. Overall, our results show that the use of DigestSea® containing MSP®$_{LIPIDS}$ helps maintain a high level of egg production and lower hepatic steatosis. In conclusion, the use of the product containing MSP®$_{LIPIDS}$ in breeding poultry production may be beneficial to maintain animal health and production levels. Further experiments are required to determine the mechanisms of action as a reduction of lipid resorption, metabolic changes, and an increased energy demand due to improving egg production.

Key words: Algae extract, liver metabolism, turkey, egg production, MSP®$_{LIPIDS}$.

1. Introduction

The digestive system of poultry breeders is highly solicited, especially in the late egg production period. Oocyte development requires transport of several exogenously produced macromolecules by the bloodstream from digestive organs as intestine, liver, kidneys, gallbladder, and pancreas. The liver is a key organ due to its important role in macronutrients and micronutrients metabolism. During egg production, the demand in lipids for yolk development increases [1-4]. The synthesis of egg yolk lipids takes place in the liver of female poultry from sexual maturity [5] around 20 weeks of age (Aviagen 2017). During yolk deposition in the egg, the liver synthetizes the major quantity of proteins which are further integrated and transformed into the yolk [6]. Besides its role in egg production, the liver is also essential for nutrient homeostasis, filtration of particles, conjugation and formation of bile and biliary excretion. Notably, primary bile acids synthetized by hepatocytes from cholesterol, enable elimination of endogenous waste and the fragmentation and emulsion of lipids from the intestine [7]. The demand for rapid growth, high nutrient intake and high metabolic rate in poultry breeders can lead to metabolic disorders as steatosis, a pathologic intracellular overload of triglycerides in hepatocytes [8]. Therefore, it is essential to support

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lipid metabolism in poultry breeders in order to maintain good health and performance. Seaweed polysaccharides and especially sulphated polysaccharides are currently studied for their different biological activities as antioxidant [9], immunomodulatory [10, 11], anticoagulant [9] and antihyperlipidemic effects [12-14]. Pengzhan et al. [12] were pioneers in the analysis of antihyperlipidemic effects of marine sulphated polysaccharides. They evidenced that two molecular weight fractions of the sulphated green algal polysaccharide ulvan improve liver metabolism in a rat model. Ulvan acts by decreasing plasma levels of triglycerides, total cholesterol, LDL-cholesterol, without affecting the one of HDL-cholesterol. More recent studies have confirmed the antihyperlipidemic activities of ulvans in mouse and rat models [13, 14]. In animal production, choline and sorbitol are frequently administered in the drinking water as well as in the feed to prevent metabolic disorders. Studies realized in dairy cows [15, 16] and in broiler breeders [17] have suggested lipotropic properties of choline as feed supplementation. Other studies on calves [18] and on dairy cows [15, 19] have shown that sorbitol supplementation in feed enhances bile production and has lipotropic properties. In poultry nutrition, choline plays an important role in metabolic function [20]. However, there is no published information on the effect of sorbitol and choline on turkeys.

MSP®$_{LIPIDS}$ is a characteristic marine sulfated polysaccharide extract from algae with action on metabolic functions. Therefore, the aim of the study was to compare the effect DigestSea®, an association of MSP®$_{LIPIDS}$ and other components including choline and sorbitol, with a complex of only choline and sorbitol (CSC) on egg production and liver lipids overload in turkeys.

2. Material and Methods

2.1 Animals

The trial was conducted during six weeks in a breeder farm with two laying buildings of turkey breeders of Aviagen B.U.T.6 genetic. One group received a liver "protector" based on a CSC at a dosage of 1 L/1,000 L of drinking water while the other group was supplemented with DigestSea® at a dosage of 0.5 L/ 1,000 L of drinking water. The ratio of choline and sorbitol in both products was 2/3 sorbitol and 1/3 choline. The dosage of choline and sorbitol administrated via DigestSea® is 25% lower than via the CSC. Both feed supplements were given during two periods, first from the 23rd week of laying during 10 days, and again the 27th week of laying during 5 days. Supplementation was performed around the end of the laying period, as it corresponds to age at which the animals are more likely to develop metabolic problems. The CSC group was constituted of 2,016 animals and the DigestSea® group of 2,110 animals.

2.2 Zootechnical performance

The egg production was monitored daily in each group before the trial (from week 1 to week 22 of laying) and during the trial (from the start of week 23 to the end of week 28 of laying).

2.3 Histology

Histological analyses were performed at the end of the trial on 21 livers for the CSC group and 22 livers for the DigestSea® group, at 28 weeks of laying. Two sections were analysed per liver, one section containing a portal area and another excluding the portal area. After staining with haematoxylin and eosin (H&E), they were observed under a Nikon Eclipse 5DI microscope equipped with a Nikon DS-42-RI camera, at ×40 magnification and analysed for hepatocytes, Küppfer cells, bile duct and blood capillaries in specific portal areas and blood capillaries in global liver (Table 1). Hepatic histological criteria were rated using a scale from 0 to 4 corresponding to lack of damage to severe damage, respectively. Steatosis was evaluated by scoring lipid

droplets in hepatocytes, from no to severe steatosis.

2.4 Statistical Analysis

Statistical analysis was performed using the R software and differences between groups were tested with Mann-Whitney test and Anova test. Statistical significance was declared with $\alpha = 0.05$ and p-value of 0.05.

3. Results

3.1 Laying Performances

Before the start of the trial, from week 1 to week 22 of laying, egg production was not statistically different between the two groups ($p > 0.05$). In Aviagen Turkey technical references, the laying rate decreases from the 23rd week, due to the age of the animals. In our study, we observe a lower reduction in the laying performance of the DigestSea® group compared to the CSC group. The difference was statistically significant when taking into account the whole trial period, from week 23 to week 28. Egg production in the DigestSea® group was significantly higher by 0.5 egg ($p < 0,05$) per turkey per week compared to the CSC group (Table 2). The present results show that further improvement of egg production in turkey breeder is possible when choline and sorbitol are combined with MSP®$_{LIPIDS}$.

During egg production, the liver is highly solicited and becomes more vulnerable to metabolic disorders with increasing age of the animals. There is little published information on turkeys but hepatic steatosis in laying hens has been shown to be concomitant with reduction in egg production [8]. Therefore, it is essential to support lipid metabolism in the liver to maintain good levels of egg production and reduce mortality mainly during the last stage of laying production. In this study, distribution of DigestSea® significantly limits the decrease of egg-laying compared to CSC, over the trial period.

Table 1 Hepatic criteria for histological decisions.

	Parameters	Criteria
Hepatocyte	Size	Homogeneous/heterogeneous
	Size homogeneity	Homogeneous/heterogeneous
	Nucleus	Regular/irregular
Global liver scoring	Blood capillaries congestion	From 0 to 4 (lack of damage to severe damage)
	Leucocytosis in blood capillaries	
Portal area scoring	Bile duct oval cell hyperplasia	From 0 to 4 (lack of damage to severe damage)
	Bile duct hyperplasia	
	Blood capillaries congestion	
	Leucocytosis in blood capillaries	
	Hyperplasia of lymphoid residual tissue	

Table 2 Average egg production per turkey per week during the trial (from week 23 to week 28 of laying).

	Mean of eggs production per turkey		
Week of laying	DigestSea®	CSC	Statistical significance
23	4.99	4.23	NS
24	4.70	4.13	NS
25	4.45	4.03	NS
26	4.38	3.91	NS
27	4.21	3.74	NS
28	3.92	3.60	NS
From weeks 1 to 22	4.42	4.41	NS
From weeks 23 to 28	4.44	3.94	$p = 0.02$

3.2 Liver Histology

No difference was observed between CSC and DigestSea® groups for hepatocyte size and conformation, in the portal area as well as outside the portal area (data not shown). The hepatocyte size and conformation are homogeneous and regular in both groups. In addition, there were no statistically significant differences for the scores of Küppfer cells in global liver and hyperplasia of the lymphoid residual tissue, between the CSC and the DigestSea® groups.

Each portal area contains a bile duct, a hepatic artery branch and a portal vein branch. We observed a significant reduction in the biliary canal hyperplasia by 73% (p = 0.007, Table 3) and in blood capillaries leucocytosis (p = 0.03, Table 3) with DigestSea® in comparison with CSC. In addition, supplementation with DigestSea® significantly reduced the blood capillary congestion, (16%, p = 0.03) in global liver but not in portal areas. However, leukocytosis was significantly reduced in portal area ($p = 4.52 \times 10^{-8}$, Table 3).

3.3 Lipids Content in Liver

Three studies have shown that choline and sorbitol reduce fat accumulation in the liver and plasma of ruminants [12, 16, 19], and one has shown the same effect in broilers [17]. In the present work, histological analyses revealed significantly less severe steatosis occurring in the DigestSea® group, in comparison with the control group (CSC group, Fig. 1, p = 0.02), with limited effect on number of cases (18/22). This is due to a shift of steatosis towards less severe forms and a slight increase in the percentages of animals free of any disease (Fig. 1). These results are in line with those of Pengzhan et al. [12] who showed that Marine Sulphated Polysaccharides lower plasmatic concentrations of triglycerides, total cholesterol, LDL-cholesterol, without significantly affecting HDL-cholesterol levels.

Table 3 Histology results of liver scoring on 21 livers from the CSC group and 22 livers from DigestSea® group.

Parameters		CSC	DigestSea®	p-value Mann-Whitney
Global liver				
Blood capillaries	Congestion	1.19 ± 0.4	1.00 ± 0.0	0.03
	Leucocytosis	1.14 ± 0.4	1.00 ± 0.0	0.07
Portal area				
Bile duct	Oval cell hyperplasia	1.00 ± 0.4	0.95 ± 0.2	0.69
	Bile duct hyperplasia	0.67 ± 0.7	0.18 ± 0.4	0.007
Blood capillaries	Congestion	1.14 ± 0.4	1.00 ± 0.0	0.07
	Leukocytosis	1.10 ± 0.6	0.05 ± 0.2	4.52×10^{-8}
Hyperplasia of lymphoid residual tissue		0.90 ± 0.9	0.68 ± 0.9	0.37

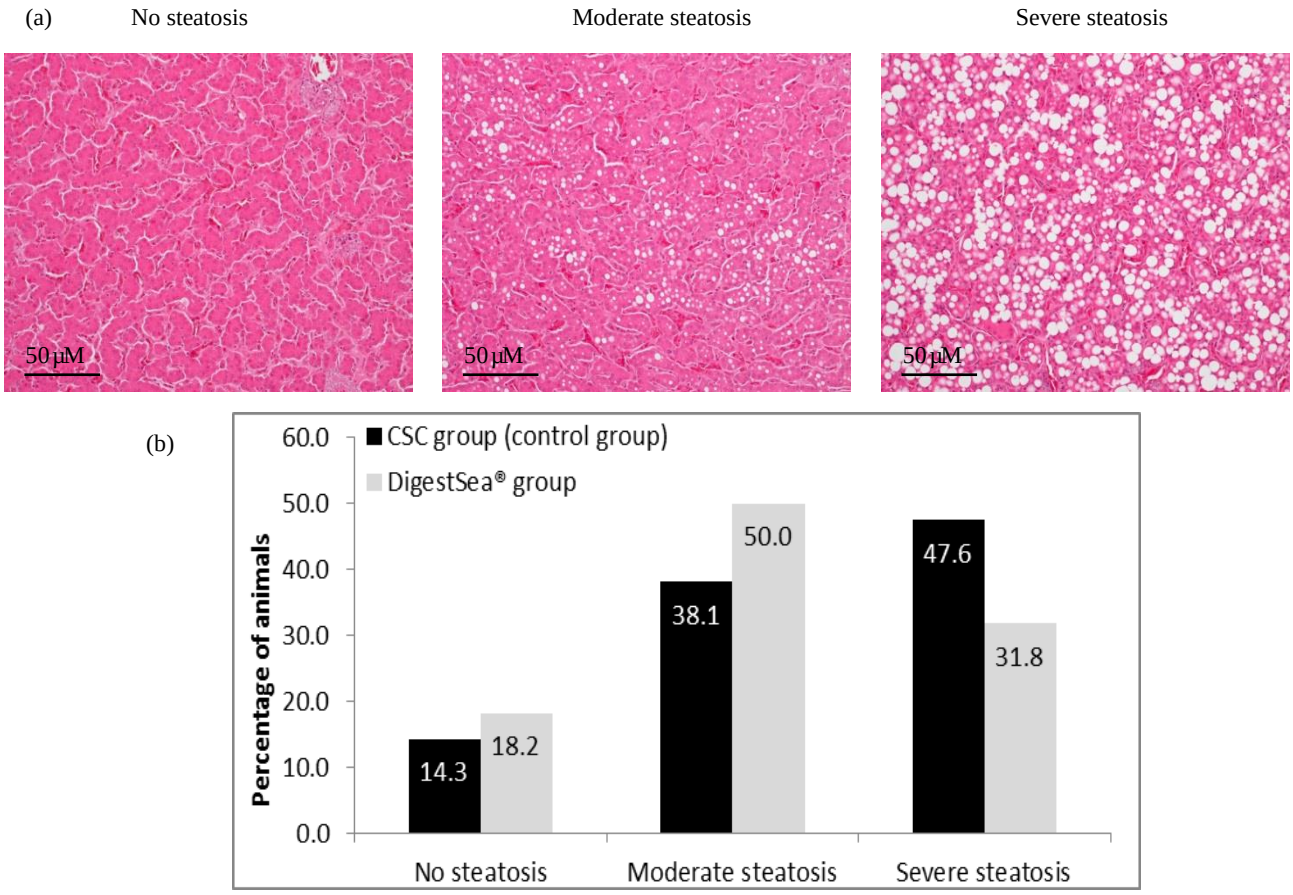

Fig. 1 (a) Histological analysis of lipid overload in the liver with haematoxylin and eosin staining (magnification ×20). Lipids droplets appear as white spots. (b) Impact of the type of supplementation on the severity of steatosis.

4. Conclusion

In conclusion, we demonstrate that a supplementation with DigestSea® results in higher egg production (0.5 egg (p < 0,05)). Regarding the liver status, we observed a global reduction of blood capillaries congestion. In the portal area, bile duct hyperplasia and leucocytosis in blood capillaries were reduced. In addition, adding DigestSea® to the regular diet results in a statistically significant reduction of lipids overload in the liver. Further experiments are required to determine if the mechanism of action is associated with as a reduction of lipid resorption metabolic changes, or improved energy efficiency … Moreover, these assumptions are not mutually exclusive and might be concomitant.

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Author's Contributions

FB, MLG, OM and PNC contributed to the conception and design of the work. FB and OM executed it. MB, NG, PNC, MLG and FB contributed to the analysis and interpretation of data. MB and NG drafted the manuscript. FB, MLG, MB, NG, OM, JD and PNC contributed to the final approval of the version for publication. All the authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All experiments involving animals have been performed in accordance with the French guidelines

and regulations.

Consent for Publication

Not applicable.

Competing Interests

FB, MLG, MB and PNC were employed by Companies Amadeite and Olmix.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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