Histological Investigations at an Intestinal Level on Laying Quails Fed with Fodders Supplemented with Different Additives (Sel-Plex, Actigen and Bio-Mos)

Teodor I. MĂLDĂRĂŞANU*[1)], Aurel ŞARA[1)], Călina CREŢA[2)], Mihai BENŢEA[1)]

[1)]University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Science and Biotechnologies, 3-5 Mănăştur Street, 400372 Cluj-Napoca, Romania
[2)]National Institute of Public Health, Territorial Center of Public Health Cluj, 6 Pasteur Street, 400349 Cluj-Napoca, Romania
*Corresponding author, email: *maldarasanu_teodor@yahoo.com*

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Abstract

The aim of this paper was to observe and highlight the possible influence of prebiotics (BioMos and Actigen) and organic Selenium (Sel-Plex), administered in the feeds of laying quails, on the intestinal mucosa and the impact it has on the production and consumption performances of the laying quails.

The research has been carried out on 132 laying quails, assigned to 4 groups, each group consisting of 33 birds; a control group fed with combined fodder without any additives and 3 experimental groups fed with additives supplemented fodders –Actigen (0.08%) in experimental group 1, Bio-Mos (0.12%) in experimental group 2 and organic Selenium (Sel-Plex –0.04%) in experimental group 3. The experimental period was 26 weeks (from the age of 6 weeks until the age of 32 weeks). The lying quails were individually weighted at the beginning, at the middle and at the end of the experimental period.

The main histological investigations that were carried out at the end of the experimental period consisted of the determination of the villi height, microvilli height and the subsequent intestinal absorption surface; also the body mass evolution, laying intensity and feed conversion were monitored. The birds in all the groups were kept in the same rearing conditions, throughout the entire experimental period.

The recorded results confirm the positive influence of the used prebiotics (Bio-Mos and Actigen) and organic Selenium (Sel-Plex) on the intestinal mucosa by increasing the nutrient absorption surface thus explaining the improvement of the production performances and feed conversion in all the experimental groups, compared to the control.

Keywords: *intestinal villi, microvilli, laying quails, organic Selenium, Sel-Plex, Bio-Mos, Actigen*

INTRODUCTION

Prebiotics are substances of organic or inorganic origin, both natural and synthesized that have the purpose of promoting the development and multiplying of prebiotic microorganisms in the digestive tract, thus contributing to a good health status and to the increase of the productive performances (Şara and Benţea, 2012). Prebiotics also enhances the nutrient absorption, of the macro an microelements, through the intestinal wall (Pop, 2006; Chen *et al*., 2004), subsequently increasing their availability for the maintenance and regenerative needs of the organisms and also for reproduction.

Bio-Mos and Actigen are oligosaccharides based prebiotics, manufactured by Alltech®, by fermenting sugars using the *Saccharomyches cerevisiae* yeast.

It has been studied that the use of some oligo-saccharides represents a method of manipulating the intestinal microbiota and prevent the on setting of potentially pathogen bacteria (Gibson *et al*., 2003) or to stimulate the absorption of minerals, specially Calcium and Magnesium (Roberfroid *et al*., 2000).

Bio-Mos and Actigen belong to the mannan-oligosaccharides category, derivates of the mannans found on the cell surface, that act as high affinity binders thus offering competitive attaching sites for certain categories of bacteria (i.e *Escherichia coli* and *Salmonella sp.*). The Gram-negative bacteria attach themselves to the MOS instead of attaching to the epithelial cells of the intestine, thus transiting the intestine without colonizing it (Şara, 2006).

Several researches carried out worldwide indicate that the Bio-Mos or Actigen treatment positively influences the performances of broilers and laying poultry, regarding both quantity and quality of production, due to the curative properties that the two substances have on an intestinal level, by boosting the immune system and maintaining the health status.

Yang *et al.* (2007), by administering Bio-Mos (2g/kg) in chicken infected with *E. coli*, observed a significant reduction of tissue associated coliforms in the first week, compared to the control group. Loddi *et al*. (2004), administering Bio-Mos on broiler chickens reported the improvement of the feed conversion ratio and an increase of villi height. Janjeeiae *et al*. (2007), Gracia *et al*. (2004) and Dimovelis *et al*. (2004), administering Bio-Mos to poultry reported an improvement of the feed conversion and a subsequent increase of production.

Gernat (2011), administering Actigen in broiler chickens reported superior results compared to the control group, the use of Actigen leading to an improvement of the FCR, reduced intestinal lesions and an enhanced immune system. Olejniczak and Nollet (2011), Haese *et al*. (2011), administering Actigen on broiler chickens noted a superior weight gain, a lower feed consumption and FCR and the decrease of mortality.

Sel-Plex, or organic Selenium, is manufactured by Alltech® and is obtained from a strain of *Saccharomyches cerevisae*, being the first form of organic Selenium approved by the European Union.

Organic Selenium (Se-Met- selenomethionine) is actively absorbed in the intestine as an amino-acid participating in the same processes as methionine. The chemical similarities between selenomethionine and methionine allow the body to use them alternatively in protein synthesis. Thus, the formation of Selenium deposits in the organism can take place (especially in muscle tissue), reserves that are later used in times of stress, when the Selenium requirements are growing and the feed consumption diminishes.

Researches carried out worldwide showed that the use of Sel-Plex determined the improvement of the productive performances in poultry while decreasing the mortality rates.

Pan *et al.* (2004) and Stanley *et al.* (2004), after administering organic Selenium (Sel-Plex) on laying hens, reported improvements of the body weight, feed conversion, egg production and egg weight. Neylor *et al*. (2000) used diets containing organic Selenium and found that by increasing the Selenium percent in feeds, the feed efficiency is significantly improved. The positive effects of Sel-Plex on the FCR were also confirmed by Edens *et al*. (2001).

The major benefit of using Sel-Plex in chicken meat production includes the improvement of weight, FCR, the strengthening of the immune system and the decrease of mortality (Anciuti *et al*., 2004; Edens and Gowdy, 2004)

MATERIALS AND METHODS

The research was carried out on 132 laying quails, randomly assigned to 4 experimental groups, 33 birds/ group, over a period of 26 weeks (02.06.2012 – 30.11.2012).

The birds were housed at the experimental location 1 week before the start of the laying period and one week prior to the beginning of the experiment, when the birds were 35 days old. This period allowed the bird a period of transition to the new environment and to the feeding regime and to avoid a delay of the laying period caused by the transport-induced stress.

The 4 groups (1 control, and 3 experimental groups) were housed in special cages designed for laying quails (dimensions being Lxlxh = 90x50x25 cm); the cages were positioned at the same height, had the same environmental conditions and received the same care throughout the experiment.

Water and feeds were constantly available for all groups. The combined fodder used in this experiment had the following composition: corn

37.5%, wheat 10%, soy bean meal 24 %, sun flower meal 13%, corn gluten 2.5%, vegetable oil 2.8%, calcium carbonate 7.5%, mono-calcium phosphate 1.2%, L-lysine0.075%, methionine 0.025%, vitamin-mineral premix 1% and salt (NaCl) 0.4%. This fodder was the base fodder that was administered to the control group and was later supplemented for the experimental groups as follows: group 1E–0.08% Actigen, group 2E–0.12% Bio-Mos and group 3E–0.04% Sel-Plex.

The environmental temperature was 23-24°C and was maintained using 2 ventilators and a heater connected to a temperature-dependant programmable power source.

In order to better understand the mechanisms that contribute to the improvement of the quantity and quality parameters in laying quails, histological test were carried out on 5 birds from each group to determine the height of the villi and microvilli representing the nutrient absorption area in the intestine. Also, during the experimental period, body mass evolution, laying intensity and feed conversion were also recorded.

RESULTS AND DISCUSSION

Even though, at the beginning of the experiment, all the groups has the same initial weight, at the middle and at the end of the experimental period, significant differences were recorded between the experimental groups and the control group, regarding the body mass (*Tab. 1*). At the middle of the experiment, a mean body weight of 261.87±5.75g was recorded in group 1E, 262.12±7.39g in group 2E and 263.06±6.33g in group 3E, compared to the control group, were a mean body weight of 250.65±6.02g was recorded.

At the end of the experimental period, the control group had almost the same body weight that was recorded at the middle of the experiment (250.00±5.48g); the experimental groups continued to accumulate body mass, the recorded values being of 281.43±5.27g in group 1E, 267.81±6.42g in group 2E and 285.06±5.22g in group L3E. Significant differences have been recorded between groups 2E and the control group regarding the body mass recorded at the end of the experimental period; the differences recorded between groups 1E, 3E and the control group were very significant.

As can be seen in *Table 2* the recorded values regarding the laying intensity indicate an improvement in the experimental groups (1E -80.78±2.73, 2E -78.46±2.89 and 3E -83.62±2.76) compared to the control group (70.17±2.17). Group 1E (that received Actigen) had a 15.12% ($p<0.05$-significant) increase compared to the control group, group 2E (Bio-Mos) 11.81% ($p<0.05$-significant) increase and group 3E (Sel-Plex) had 19.16% ($p<0.01$-distinctly significant) increase, compared to the control group.

Regarding the feed conversion ratio and the average feed consumption/egg, the recorded differences were distinctly significant (L2E-$p<0.01$) and very significant (L1E and L3E-$p<0.001$) in favor of the experimental groups. The quails from the experimental groups had a lower consumption of fodder/egg (35.77±1.10g in group 1E, 36.87±1.17g in group 2E and

Tab. 1. Body weight of quails at the middle and at the end of the experimental period.

Issue		Control group	Group 1E (Actigen)	Group 2E (Bio-Mos)	Group 3E (Sel-Plex)
N		33	33	33	33
Initial weight (g) (3 weeks of age)	X±xs	183.15±3.35	183.24±3.45	182.42±3.56	183.27±5.18
	V%	10.54	10.81	11.23	16.26
N		29	32	31	33
Weight – middle of experiment (g) (19 weeks of age)	X±xs	250.65±6.02	261.87±5.75	262.12±7.39	263.06±6.33
	V%	12.93	12.42	15.70	13.83
N		28	32	31	33
Final weight (g) (32 weeks of age)	X±xs	250.00±5.48	281.43±5.27***	267,81±6.42 *	285.06±5.22 ***
	V%	11.60	10.59	13.36	10.52

***- very significant P<0.001; *- significant P<0.05; ns– non-significant P>0,05; V%- variability; X– media; xs– standard deviation; n– number of birds.

Tab. 2. The mean values of the production and consumption indices in laying quails throughout the experimental period.

Issue		Experimental groups			
		M	Group 1E (Actigen)	Group 2E (Bio-Mos)	Group 3E (Sel-Plex)
Total weight gain	g/26 weeks.	66.82	98.19	85.39	101.79
	%	100	146.94	127.79	152.33
Laying intensity (%)	Absolute	70.17±2.17	80.78±2.73*	78.46±2.89*	83.62±2.76**
	Relative	100.00	115.12	111.81	119,16
Feed conversion ratio/ egg	g	43.09 ± 1.19	35.77 ±1.10***	36.87±1.17**	34.19±1.30***
	%	100	83.01	85.57	79.35

*- p<0.05- significant ; **- p<0.01- distinctly significant; ***- p<0.001- very significant.

Tab. 3. The height of the intestinal villi in laying quails (values are average of 10 determination/ sample, µm).

	Control group	Experimental groups		
Samples	**M**	**Group 1E**	**Group 2E**	**Group 3E**
n	5	5	5	5
X±xs	1101.38±24.39	1391.66±29.20 **	1627.45±60.69 ***	1511.46±46.89 ***
V%	4.95	4.69	8.34	6.94

- p<0.01-distinctly significant; *- p<0.001-very significant; V%-variability; X–average; xs–standard deviation; n–number of samples.

Tab. 4. The height of the intestinal microvilli in laying quails (values are average of 10 determination/ sample, µm).

	Control group	Experimental groups		
Probe	**M**	**Group 1E**	**Group 2E**	**Group 3E**
n	5	5	5	5
X±xs	2.70 ± 0.13	3.07 ± 0.18	3.20 ± 0.24	3.18 ± 0.20
V%	10.54	13.43	16.78	14.16

V%- variability; X– mean; xs–standard deviation; n– number of samples;
L1E–1st experimental group; L2E–2nd experimental group; L3E–3rd experimental group.

34.19±1.30g in group 3E) compared to the control group (43.09±1.19g). The feed consumption for an egg was 16.99% lower in the experimental group 1E (0.08% Actigen) compared to the control group; 14.43% lower in the experimental group 2E (0.12% Bio-Mos) and 20.65% lower in the experimental group 3E (0.04% Sel-Plex).

The results regarding the improvement of the production and consumption indices of laying poultry, by dietary supplementation using organic Selenium (Sel-Plex) are also confirmed by Stanley *et al.* (2004), Pan *et al*. (2004), Şara *et al*. (2008); the positive effects of Bio-Mos are also confirmed by Janjeeiae *et al*. (2007), Kocher *et al*. (2005) and the favorable effects of Actigen have also been described by Lea *et al.* (2011) and Munyaka *et al.* (2011).

To underline the effects the prebiotics (Bio-Mos and Actigen) and the organic Selenium (Sel-Plex) have at an intestinal level and subsequently on the production and consumption indices, histological investigation have been carried out, consisting of determinations of the villi and microvilli height. *Table 3* shows the data regarding the height of the intestinal villi at the end of the experimental period, in the quails from all the experimental groups, their aspect being shown in *Figure 1*.

The measurements carried out on the height of the intestinal villi of quails show increases of the villi structures in all the experimental groups (1391.66±29.20µm in group 1E, 1627.45±60.69µm in group 2E and 1511.46±46.89µm in group 3E) compared to the control group (1101.38±24.39µm); the result recorded in the experimental groups were 26.35%, 47.76% and 37.23% higher than the one recorded in the control group. *Table 4* presents the values regarding the height of the intestinal microvilli in laying quails, their aspect being shown in *Figure 2*. Group 1E (Actigen) had a lower mean value of the microvilli height (3.07±0.18 µm) 13.7% higher than the values recorded in the control group (2.70±0.13 µm). Group 2E (Bio-Mos) had a mean value of 3.20±0.24 µm, 18.51% higher than the control group; group 3E (Sel-Plex) had a mean value of 3.18±0.20 µm, 17.77% higher than the control group.

The data recorded from the histological investigations confirm the positive influence of the used additives (Actigen, Bio-Mos, Sel-Plex) on he intestinal morphology, regarding the nutrient absorption area. Thus, all the recorded results regarding production that were recorded in the experimental groups can be explained by the improved and accelerated nutrient absorption. The results found by the histological investigations are confirmed by the works of other authors that researched the influence of these additives on poultry. Bonos *et al*. (2010, 2011) found that the use of mannan-oligosaccharides and prebiotics (MOS) on laying quails can positively influence the intestinal function by improving the height, uniformity and integrity of the intestinal villi. Loddi *et al.* (2004) investigated the effects of Bio-Mos on performances and intestinal morphology in broiler chickens and reported that the use of Bio-Mos improved the feed conversion ration at the same time increasing the height and width of the intestinal villi. Papazyan and Surai (2004) found that the dietary supplementation using Sel-Plex can improve the intestinal morphology and subsequently the nutrient absorption area, results also confirmed by Dibner (2000).

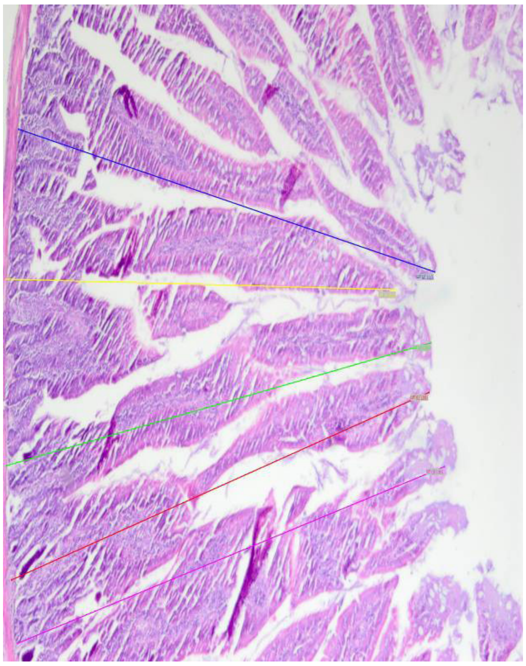

Fig. 1. Intestinal villi in quail (Olympus BX51) (Hematoxylin Eosin, 4x magnification)

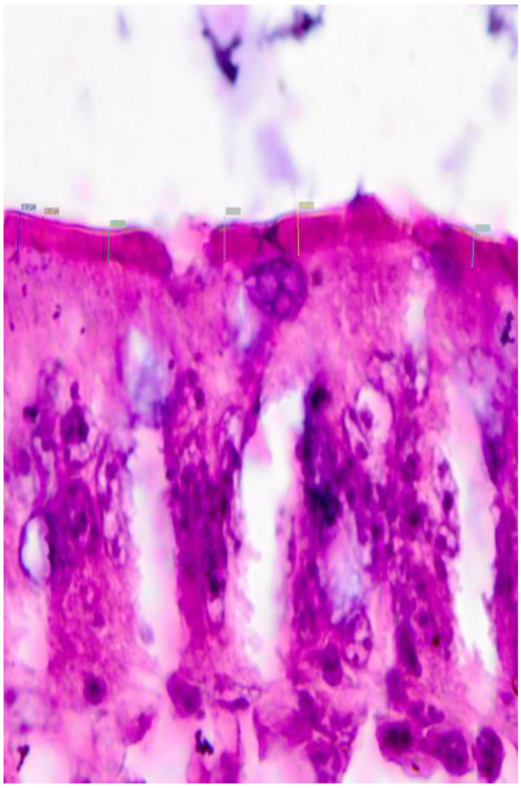

Fig 2. Intestinal microvilli in quails (Hematoxylin Eosin, 100x magnification)

CONCLUSION

The histological investigations carried out at the end of the experimental period on laying quails showed that the use of prebiotics (Actigen and Bio-Mos) and organic Selenium (Sel-Plex) led to a better development of the intestinal villi and microvilli thus increasing the nutrient absorption area leading to an improved nutrient assimilation that translated to superior body weight, productions and feed conversions in all the experimental groups, compared to the control group.

Based on the results recorded, we recommend the use of Actigen and Bio-Mos prebiotics and Sel-Plex organic Selenium in laying quails.

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