

## **Effect of probiotic inclusion in different levels of barley substitution for corn diets on laying hen's histological changes of duodenum**

A. H. Mahdavi, H. R. Rahmani\* and J. Pourreza, Department of Animal Science, Isfahan University of Technology, 84156 Isfahan, Iran, E-mail: [mahdavi@ag.iut.ac.ir](mailto:mahdavi@ag.iut.ac.ir)

### **Abstract**

The effect of probiotic (0, 400, 1000 and 2000 gr Bioplus 2B ton<sup>-1</sup> feed providing 0, 1.28×10<sup>6</sup>, 3.2×10<sup>6</sup> and 4.6×10<sup>6</sup> cfu gr<sup>-1</sup> feed concentration) in different levels of substitution of barley for corn diets (0, 50 and 100 percent) on histological changes of duodenum was investigated on two hundred forty white leghorn Hy-Line, W-36 strain. Using different levels of substitution of barley for corn showed highly significant increase (P<0.01) in goblet cell numbers and epithelium surface folds of villus and significant increase (P<0.05) in damaged apical cells of villus. Addition of different levels of probiotics caused the highly significant increase (P<0.01) in goblet cell numbers and significant increase (P<0.05) in destroyed apical cells of villus. Feeding the third and fourth levels of probiotic caused significant proliferation of lymphatic system in the lamina propria layer, with hyperplastic conditions.

### **Introduction**

*The concept of “gut health” is complex and, at present, is an ill-defined notion. There are three major components of “gut health”, namely the diet, the mucosa and the commensal flora. The mucosa is composed of the digestive epithelium, the gut-associated lymphoid tissue (GALT) and the mucus overlying the epithelium. The GALT, commensal bacteria, mucus and host epithelial cells interact with each other forming a delicate and dynamic equilibrium within the alimentary tract that ensures efficient functioning of the digestive system (Montagne et al, 2003). Dietary fibre (DF) interacts both with the mucosa and the microflora, it has an important role in the control of “gut health”. In general, DF ingestion leads to increased size and length of the digestive organs, including the small intestine, caecum and colon of chickens (Iji et al., 2001). Probiotics are defined as microbial cell preparations that have a beneficial effect on the health and wellbeing of the host (Fuller, 1989). Experimental data indicate that some probiotics stimulate mucosal immunity, display a trophic action on the mucosa, reduce mucus degradation and interact with mediators of inflammation (Fioramonti et al., 2003).*

### *Materials and Methods:*

*During the 12 weeks of the experiment (28-39 weeks-old), two hundred and forty white leghorn hens Hy-Line, W-36 strain were randomly allocated in a factorial arrangement (3×4) based on a completely randomized design considering 12 treatments with 4 replicates and 5 samples in each. Both supposed factors including 4 levels of probiotic (0, 400, 1000 and 2000 gr Bioplus 2B ton<sup>-1</sup> feed providing 0, 1.28×10<sup>6</sup>, 3.2 ×10<sup>6</sup> and 4.6× 10<sup>6</sup> cfu gr<sup>-1</sup> feed concentration) and 3 levels of barley substitution for corn (0, 50 and 100 percent). Bioplus 2B, contained 2 strains of bacilli. Bacillus subtilis (CH201) and Bacillus licheniformis (CH200) with a minimum of 3.2× 10<sup>9</sup> cfu gr<sup>-1</sup> of the product.*

*At the end of the trial, one hen was randomly sacrificed from each replicate for studying histological changes in duodenum. A 2-cm length of descending duodenal segment was excised for light microscopic observations with standard procedure. Data were analyzed using the General Linear Models (GLM) procedure of Statistical Analyses Systems (SAS, 1999).*

### *Results and Discussion:*

Using different levels of barley substitution for corn motivated highly significant increase ( $P<0.01$ ) in goblet cell numbers, epithelium surface folds of villus and significant increase ( $P<0.05$ ) in damaged apical cells of villus. Compatibility of apical cells with different rations were definitely obvious with different levels of barley substitution particularly at second and third levels. The apical cells were refurbished by increasing the folding the villus. This manner was accompanied by increasing the goblet cell proliferation, which together protected the gut wall by unstirred water layer. The effect of DF on epithelial morphology and cell turnover is variable, and depends on the physico-chemical characteristics of the DF, their level of incorporation in the diet, the duration of ingestion, the animal species and age, and the site in the intestinal tract. Montagne et al, (2003) suggested that effect of DF on intestinal epithelial anatomy and structural development seems to be dependant on the ability of particular DF to increase digesta viscosities. Although some authors suggested that treatments decreasing the digesta viscosity through the use of exogenous enzymes have reduced the deleterious effect of fiber on the small intestine mucosa (Simon, 1998), but addition of  $\beta$ -glucanase enzyme in production period of laying hens in this trial had no positive effect on duodenum health. Blottieres et al., (1999) suggested that the increase of crypt-cell proliferation induced by DF can also be explained by the trophic effect of SCFA and especially butyrate, acting through mechanisms that are still incompletely understood.

The probiotic affected the cellular changes but in another from. Probiotic supplementation had almost damaged the apical cells significantly ( $P<0.05$ ) and increased the goblet cells highly significant ( $P<0.01$ ) without any effect on folding of villus. Using of third and fourth levels of probiotic caused the lymphatic system in the lamina propria layer be significantly proliferated, with hyperplasia conditions, which is more likely similar to defensive reaction against antigens. These reactions might be also related to response of animal cells to microbial enzymes such as phospholipase  $A_2$ , because increasing the level of probiotic at third and fourth levels damaged the tissue more seriously.

Table1: Interaction of different percentages of barley substitution for corn and probiotic levels on histological changes of duodenum

Barley substitution for corn levels (%)	Probiotic levels (cfu gr <sup>-1</sup> feed)	goblet cell numbers	Villus epithelium surface folds	villus destroyed apical cells
0	0	-	-	-
0	1.28×10 <sup>6</sup>	++	-	++
0	3.2 ×10 <sup>6</sup>	+++	++	+++
0	4.6× 10 <sup>6</sup>	++++	++	+++
50	0	-	++	+++
50	1.28×10 <sup>6</sup>	++	++	++
50	3.2 ×10 <sup>6</sup>	+++	++	++++
50	4.6× 10 <sup>6</sup>	+++	++	++++
100	0	+	++++	+++
100	1.28×10 <sup>6</sup>	+++	++++	++
100	3.2 ×10 <sup>6</sup>	++++	++++	+++++
100	4.6× 10 <sup>6</sup>	+++++	++++	+++++
Sources of variation				
Barley substitution for corn levels (B)		0.0012**	0.002**	0.0429*
Probiotic levels (P)		0.0001**	ns	0.0229*

\*Significant at  $P<0.05$ , \*\*Significant at  $P<0.01$ , (-) No effect, (+) Least effect, (++) Less effect, (+++) Moderate effect, (+++++) Serious effect, (+++++) Very serious effect.

## References

1. **BLOTTIERES, H. M., CHAMP, M., HOEBLER, C., MICHEL, C. and CHERBUT, C.** (1999) Production and digestive effects of short chain fatty acids. *Science Aliment.* **19**: 269–290.
2. **FIORAMONTI, J., THEODOROU, V. and BUENO, L.** (2003) Probiotics: what are they? What are their effects on gut physiology? *Best Practice & Research Clinical Gastroenterology* **17**: 711–724.
3. **FULLER, R.** (1989) Probiotics in man and animals. *Journal of Applied Bacteriology* **66**: 365–378.
4. **IJI, P. A., SAKI, A. A. and TIVEY, D.R.** (2001) Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. *Animal Feed Science and Technology* **89**: 175–188.
5. **MONTAGNE, L., PLUSKE, J. R. and HAMPSON, D. J.** (2003) A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology* **108**: 95–117.
6. **SAS Institute.** (1999) **SAS User's Guide. Release 8.2 Ed. SAS Institute Inc. Cary. NC.**
7. **SIMON, O.** (1998) The mode of action of NSP hydrolyzing enzymes in the gastrointestinal tract. *Journal of Animal and Feed Science* **7**: 115–123.