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RESEARCH ARTICLE

# The Effect of *Saccharomyces cerevisiae* on the Morphological and Histochemical Characteristics of the Duodenal Mucosa in the Rabbit [1]

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## Summary

The aim of this study was to determine the effect of *Saccharomyces cerevisiae* (SC) on the morphological and histochemical properties of duodenum and duodenal submucosal glands of rabbits. Twenty 5-6 weeks old male New Zealand White Rabbits were obtained from the experimental animal laboratory of Uludag University, Bursa. The rabbits were divided randomly into two groups for 90 day. The first group (control group) received the basal diet, the second group (SC group) received basal diet supplemented with *Saccharomyces cerevisiae* at a level of 3 g/kg of feed. Duodenal tissue were taken at the end of the experiment from duodenum of animals and fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were stained for localizing and characterizing glycoproteins (GPs) and morphometric measurements. In this study, the total mucosa, villus heights and gland depth of the duodenum were found to be longer than those of the control group in the SC group. However, duodenal crypt depth was greater in the duodenum of control groups, but no significant difference between the groups. The Goblet cells showed similar reaction in the both groups. Brunner glands were similar stained with AB pH 1, pH 2.5 and PAS/AB pH 1 in the both groups. However, they showed stronger positive reaction with PAS and PAS/AB pH 2.5 staining in the SC group compared with the control. In conclusion, the addition of SC to the diet of rabbits increased the total mucosa, villus height, and gland depth. However, the addition of SC also little affected the histochemical features of the duodenum by increased the secretion neutral and acidic mucins in the Brunner's glands. Therefore, it may be proposed that higher doses of *Saccharomyces cerevisiae* may be used for digestive health.

***Keywords:*** *Histology, Histochemistry, Rabbit, Saccharomyces cerevisiae, Duodenum*

# *Saccharomyces cerevisiae*'nin Tavşan Duodenumunun Morfolojik ve Histokimyasal Özellikleri Üzerine Etkisi

## Özet

Çalışma, tavşanların duodenum ve submukozal bezlerinin morfolojik ve histokimyasal özellikleri üzerine *Saccharomyces cerevisiae*'nin (SC) etkisini belirlemek amacıyla planlanmıştır. Çalışmada Uludağ Üniversitesi deney hayvanları uygulama ve araştırma merkezinden temin edilen 20 adet, 5-6 haftalık, erkek Yeni Zelanda Beyaz Tavşanı kullanıldı. Hayvanlar rastgele iki gruba ayrılarak 90 gün boyunca bakım ve beslemesi yapıldı. I. grup (kontrol grubu) bazal diet ile beslenirken, II. grup (SC grubu) 3 g/kg dozda *Saccharomyces cerevisiae* ilave edilen bazal dietle beslendi. Deneyin sonunda hayvanların duodenumlarından örnekler alındı, %10 nötral formol ile tespit edildi ve parafine gömüldü. Kesitler, glikoproteinlerin (GP) lokalizasyonu, karakterizasyonu ve morfolojik ölçümler için boyandı. Deney sonrasında, SC grubunda duodenumun total mukoza, villus yüksekliği ve bez derinliği kontrol grubuna göre daha fazlaydı. Duodenumun kript derinliği kontrol grubunda daha fazlaydı fakat gruplar arasında istatistiksel bir farklılık gözlenmedi. Her iki grupta Goblet hücreleri benzer reaksiyon gösterdi. Brunner bezleri AB pH 1, pH 2.5 ve PAS/AB pH 1 boyamaları bakımından iki grupta benzer reaksiyon gösterirken, PAS ve PAS/AB pH 2.5 boyamaları kontrol grubuna göre SC grubunda güçlü pozitif reaksiyon gösterdi. Sonuç olarak, tavşan diyetine ilave edilen SC duodenumun total mukoza, villus yüksekliği ve bez derinliğini arttırdı. Bununla birlikte SC, Brunner bezlerinde asidik ve nötral musinlerin sekresyonunu arttırarak duodenumun histokimyasal özelliğini çok az etkiledi. *Saccharomyces cerevisiae*'in daha yüksek dozlarının sindirim sağlığı için kullanılabileceği düşünülmektedir.

***Anahtar sözcükler:*** *Histoloji, Histokimya, Tavşan, Saccharomyces cerevisiae, Duodenum*

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# INTRODUCTION

Probiotics are preparation of live microorganisms (like *Lactobacillus acidophilus, Streptococcus faecium* and *Saccharomyces cerevisiae*), which have beneficial effects on the health of the human or animal when administered adequately [1]. Several commercial formulations of *Saccharomyces cerevisiae* (SC) or its derivatives are used as prebiotics or probiotics in animal diets or feed additives. There have been numerous studies in humans and animals on the ability of probiotics to change the types and numbers of gut microflora [2-4]. Probiotics inhibit the growth of pathogenic microorganisms and provide digestive enzymes, a desirable effect for the host, and as a result changes in the intestinal microflora, antibiotic production, and synthesis of lactic acid leading to lowering of the intestinal pH, adhesion or colonization to intestinal mucosa and prevention of ammonium synthesis [5,6]. However, probiotic Saccharomyces spp may also help to reestablish a normal gut function after long term antibiotic therapy [7]. *Saccharomyces* spp have protective effects, and specific activities, against various enteric pathogens [8].

Currently there are only two probiotics approved for rabbits in the EU. One of them is bacterial, *Bacillus cereus* var. toyoi, the other is yeast, *Saccharomyces cerevisiae* strain NCYC Sc47 [9]. Studies with probiotics in rabbits are less than in other monogastric farm species. Because the rabbits have a high prolific nature, rapid growth rate, feed efficiency and economic management, they have been used as material in the present study.

However, there are no conclusive data on the effects in the duodenum when live yeast is used as a dietary supplement. Therefore, the objective of the present study was to assess the effects of a dietary supplement of *Saccharomyces cerevisiae* (live yeast culture) on the morphometric characteristics and histochemical activity of the duodenum in the rabbits.

# MATERIAL and METHODS

### *Animals and Feeding*

Twenty, five-six weeks old male New Zealand White rabbits with a mean body weight of 1.000 g were included in this study. The rabbits were housed individually in metal cages, feed and water were offered *ad libitum* to the rabbits throughout the 90-day trial. After adaptation, rabbits were equally divided in two groups. The first group of animals (basal diet group) was fed with a standard feed. Basal diet (pelleted) was formulated to contain 2.500 kcal ME/kg metabolizable energy, 16% crude protein and was designed to meet maintenance requirements according to the National Research Council (NRC). The second group (SC diet group) was fed with *Saccharomyces cerevisiae* live yeast culture (Yea Sacc, Altech, Nicholasville: $1x10^9$ CFU $g^{-1}$) added at concentration 3.0 g/kg into the basal diet *(Table 1)*. The experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (2010 09/01).

### *Histology and Morphometric Analysis of the Duodenum*

At the end of the experimental period the rabbits were slaughtered and duodenum samples approximately 3-4 cm below the pylorus were taken out. Samples were fixed in 10% neutral buffered formalin. The routine histological methods were applied to the samples and embedded in paraffin. Five µm thick sections were cut from paraffin blocks, mounted on slides, and dried overnight. After dewaxing and rehydration, sections were stained by the Crossman's triple stain for morphometric examination and duodenal mucosa morphology. Moreover, histochemical techniques were used to distinguish the duodenal glycoproteins (GPs).

The villus height, the depth of the crypts, glands and

***Table 1.*** *Composition of the basal diet fed to rabbit*[a]
***Tablo 1.*** *Tavşanların bazal diyet kompozisyonu*[a]

| Ingredients | Usage Rate, % |
|---|---|
| Barley | 30.00 |
| Corn | 17.61 |
| Rice bran | 10.00 |
| Corn bran | 3.60 |
| Alfalfa meal | 25.00 |
| Soybean meal | 10.83 |
| Marble dust | 1.40 |
| Dcp | 0.28 |
| Salt | 0.80 |
| Methionin | 0.09 |
| Anticoccidial | 0.03 |
| Vitaminpremix[b] | 0.25 |
| Antioccidial | 0.03 |
| ***Calculated analysis (% DM)*** | |
| Dry matter % | 88.89 |
| Crude fiber %[c] | 10.95 |
| Crude protein %[c] | 16.00 |
| Ether extracts %[c] | 3.52 |
| Ash | 7.68 |

[a] *Yeasacc containing $1x10^9$ CFU of Saccharomyces cerevisiae was added to the basal diet at 3.0 g/kg to provide dietary treatments,*
[b] ***Premix:*** *Vit A 4.800.000 IU, Vit D 800.000 IU, Vit E 14.000 mg, Biotin 18 mg, CH-CL 50.000 mg, Folic acid 400 mg, Niacin 8.000 mg, Pant.Acid 4.000 mg, Riboflavin 2.800 mg, Thiamin 1.200 mg, Pyridoxine 2.000 mg, Vit K 1.600 mg, Zinc 24.000 mg, Iron 2.000 mg, Iodine 400 mg, Manganese 32.000 mg, Selenium 60 mg, Copper 24.000 mg*
[c] *Based on % Dry Matter*

total mucosa were measured and micrographs were taken with Nikon 80i microscope. The villus height was measured from the villus tip to villus-crypt junction level for randomly 5 villi per section. Crypt depth was measured from the villus-crypt junction to the lower limit of the crypt was estimated for 5 corresponding crypts per section [10,11]. The thickness of Brunner's glands was measured from the lower limit of the crypt to the tunica muscularis. Total mucosa thickness was measured from top of the villus to the lower limit of the crypt. *Fig. 1* illustrates the measurements that were made.

***Histochemistry***

Sections were stained with histochemical procedures for glycoproteins (GPs) identification;

**1. PAS** (Periodic Acid-Schiff's reagent) to demonstrate neutral mucosubstance [12].

**2. AB pH 2.5** (Alcian Blue 8GX pH 2.5) to demonstrate acidic GCs with carboxylated and sulphated esters [13].

**3. AB pH 1.0** (Alcian Blue 8GX pH 1.0) to demonstrate GPs with O-sulfate esters [13].

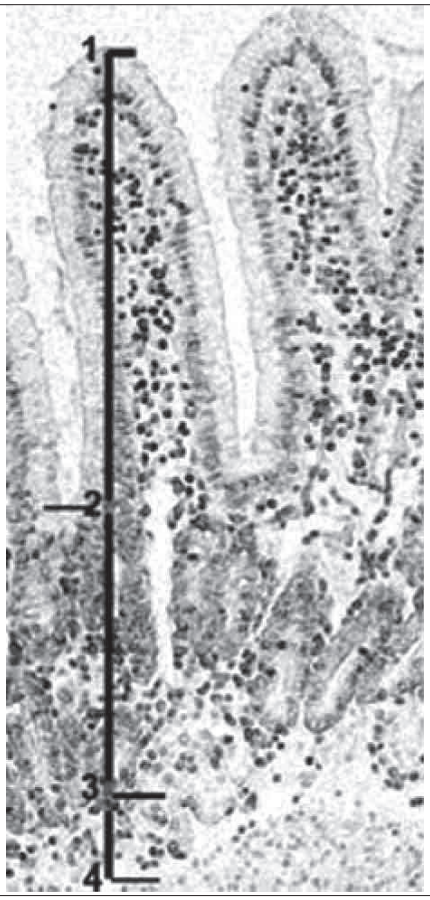


**Fig 1.** Morphological measurements in the duodenum, (1-2) villus height, (2-3) depth of crypt, (3-4) thickness of Brunner's glands, (1-3) thickness of total mucosa

**Şekil 1.** Duodenumda morfolojik ölçümler, (1-2) villus yüksekliği, (2-3) kript derinliği, (3-4) Brunner bez kalınlığı, (1-3) total mukoza kalınlığı

**4. AB pH 2.5/PAS** (Alcian Blue 8GX pH 2.5/Periodic Acid-Schiff staining) to demonstrate neutral and/or acid rich GCs [14].

**5. AB pH 1.0/PAS** to demonstrate GPs with O-sulfate esters, periodate-reactive vicinal diols, and presence of GPs with O-sulfate esters together with periodate-reactive vicinal diols [14].

All the slides were coded so that the investigator was blinded to staining for each slide and graded them according to the following scale: - no staining, + slight, ++ medium, +++ strong.

***Statistical Analysis***

Statistical analysis of results was performed by Mann Whitney U test (SPSS 16.0). Values are presented as means±SE. Group differences were declared significant at $P<0.05$.

# RESULTS

Morphology results of the total mucosa, villus height, crypt depth and gland depth are presented in *Table 2*. In this study, the total mucosa, villus heights and gland depth of the duodenum were found to be longer than those of the control group in the SC group, but there was no statistically significant ($P>0.05$) difference between groups. However, duodenal crypt depth was decreased in rabbits fed with SC compared with control rabbits but not statistically significant ($P>0.05$).

The implementation of different histochemical techniques to demonstrate the presence of GPs in the goblet cells showed a similar pattern of distribution at both groups *(Table 3)*. The Goblet cells showed a strong positive reaction with the PAS *(Fig. 2)*, and PAS/AB (pH 2.5, pH 1) staining, while no reaction with the AB staining at different pHs in both control group and SC group.

AB pH 1 technique, which allowed the identification of GPs with O-sulfate esters, showed a slightly positive reaction at Brunner Glands of both diet groups. However SC group's neutral mucosubstance in Brunner glands were

***Table 2.*** *Total mucosa, villus height, crypt depth and gland depth of duodenum in the groups (mean ± SE)*

***Tablo 2.*** *Gruplarda duodenumun total mukoza, villus yüksekliği, kript derinliği ve bez derinlikleri (mean±SE)*

| Regions of Duodenum | Group | |
|---|---|---|
| | Control | SC |
| Total Mucosa (µm) | 362.09±43.15 | 433.99±47.60 |
| Villus Height (µm) | 280.30±28.94 | 357.90±39.77 |
| Crypt Depth (µm) | 81.80±14.40 | 80.81±7.20 |
| Gland Depth (µm) | 198.75±18.73 | 225.57±17.16 |

***Table 3.*** *Histochemical staining score values of glycoproteins in the goblet cells and Brunner gland cells of duodenum in the groups*

***Tablo 3.*** *Grupların duodenumunda goblet hücreleri ve Brunner bezi hücrelerinde glikoproteinlerin histokimyasal boyanma skorları*

| Procedures | Goblet Cells | | Brunner Glands | |
|---|---|---|---|---|
| | Control | SC | Control | SC |
| PAS | +3 | +3 | +1 | +2 |
| AB pH 2.5 | 0 | 0 | +3 | +3 |
| AB pH 1 | 0 | 0 | +1 | +1 |
| AB pH 2.5/PAS | +3 | +3 | +2 | +3 |
| AB pH 1/PAS | +3 | +3 | +2 | +2 |

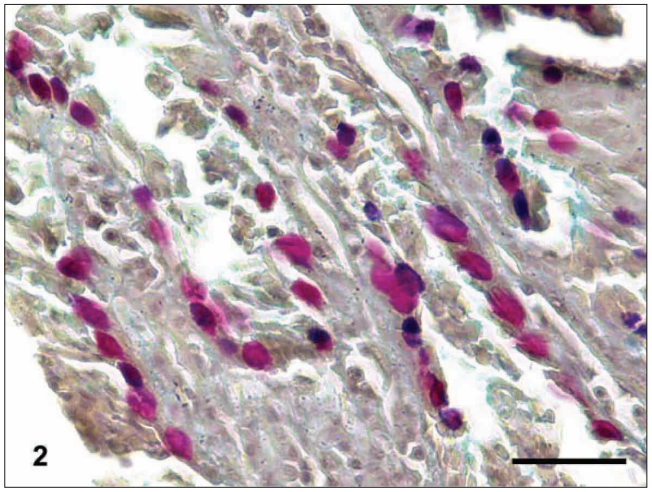


**Fig 2.** Histochemical characterization of the duodenum; Goblet cells reacted positively with PAS in the control group. PAS staining, Bar: 50 µm

**Şekil 2.** Duodenumun histokimyasal karakterizasyonu; Kontrol grubunun Goblet hücrelerinde PAS pozitif reaksiyon. PAS boyama, Bar: 50 µm

moderately stained by PAS, control groups' were showed slightly positive reaction *(Fig. 3 A,B)*. Also Brunner glands at both diet groups were strongly stained blue with AB pH 2.5 which allowed the identification of GPs with carboxyl groups. They were stained strongly positive at SC group on the other hand moderately stained at control group with PAS/AB pH 2.5; to demonstrate GPs with carboxyl groups and GPs with O-sulfate esters *(Fig. 4 A,B)*. Both diet groups were stained moderate red by PAS/AB pH 1.

## DISCUSSION

This study contains the histological and histochemical changes of duodenum after feeding rabbits with SC. In our study, we observed that total mucosa height was higher in the SC group compared with those in the control groups. This result was related with increasing villus height. However, the difference was not statistically significant. It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area [15].

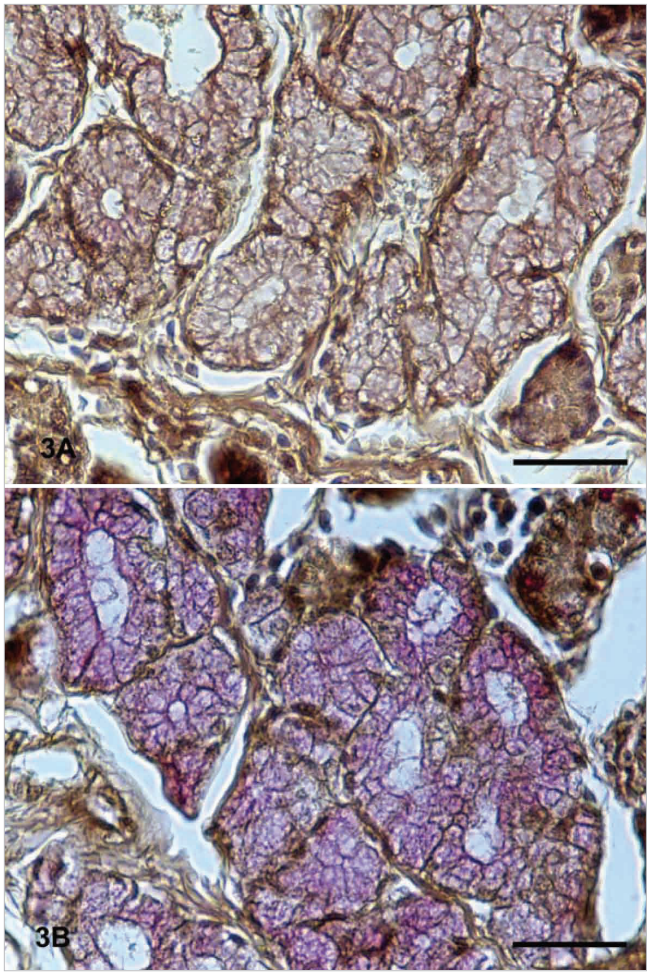


**Fig 3.** Histochemical characterization of the duodenum, PAS staining, **A-** Brunner's glands stained slightly PAS positive in control group, **B-** Brunner's glands stained moderately PAS positive in SC group, Bar: 50 µm

**Şekil 3.** Duodenumun histokimyasal karakterizasyonu, PAS boyama, **A-** Kontrol grubunun, Brunner bezlerinde zayıf PAS pozitif reaksiyon, **B-** SC grubunun, Brunner bezlerinde orta şiddette PAS pozitif reaksiyon, Bar: 50 µm

According to Buts et al.[16] *Saccharomyces* have a positive effect on the villus height. Likewise, Baum et al.[17] also found that villus length was greater in the small intestine of piglets fed yeast than controls. In addition, it was indicated that longer villi are correlated with activation of cell mitosis [18]. Hence, our results confirm this hypothesis that these yeasts could stimulate the development of the intestinal villi by an increasing cell proliferation.

In the present study, a greater villus and shorter crypts were observed in SC fed rabbits. Santin et al.[19] reported very similar results in that SC added at 0.2% of broiler diets that a reduction in crypt depth and an increase in villus height. Likewise, Bradley et al.[20] reported that crypt depth in the ileal mucosa was reduced when the broiler diet was supplemented with SC. In this study the Brunner's glands in the duodenum of the group feeding with SC was found to be higher than those in the control group but not

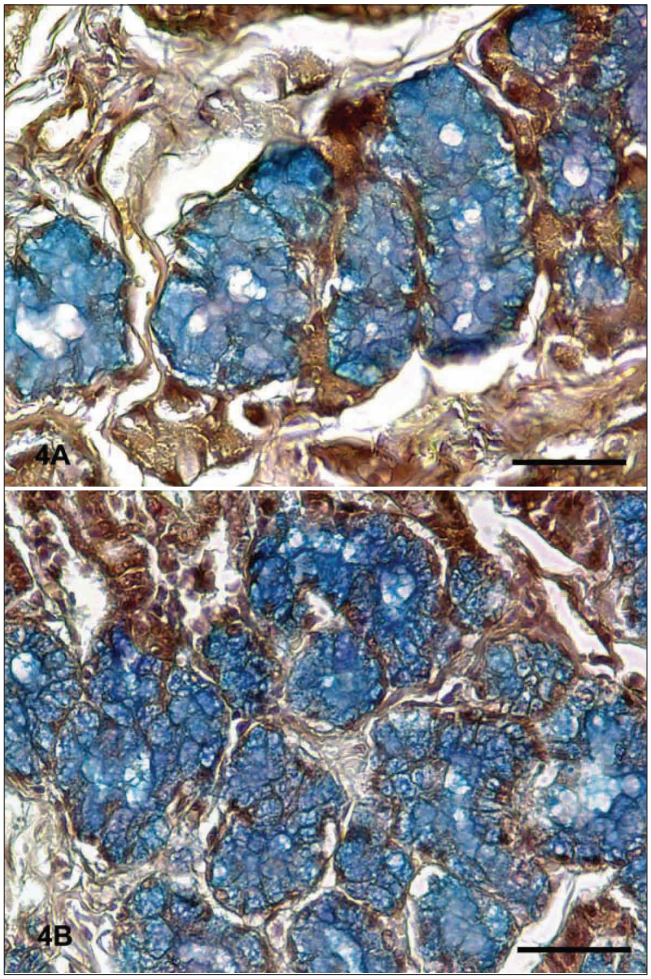


**Fig 4.** Histochemical characterization of the duodenum, AB pH 2.5/PAS staining, **A-** Brunner's glands stained moderately AB/PAS positive in control group, **B-** Brunner's glands stained strongly AB/PAS positive in SC group, Bar: 50 µm

**Şekil 4.** Duodenumun histokimyasal karakterizasyonu, AB pH 2.5/PAS boyama, **A-** Kontrol grubunun, Brunner bezlerinde orta şiddette AB/PAS pozitif reaksiyon, **B-** SC grubunun Brunner bezlerinde şiddetli AB/PAS pozitif reaksiyon, Bar: 50 µm

statistically significant. This study showed that the gland depth may be increased by SC's inducing the enlargement of the Brunner's glands.

Also, we demonstrated the presence of GPs with different histochemical techniques in the duodenal glands of the groups. In mammals, GPs layer of gastrointestinal tract protects the epithelial cells and mucosa from proteolytic enzymes invasion of enteric bacteria, bacterial and environmental toxins, and some dietary components [21,22]. This glycoprotein compounds also known as mucins which secreted by goblet cells [23]. Various authors have suggested that goblet cells contain neutral or acidic mucin glycoproteins or the combination of both types of mucin [24-26]. In classic carbonhydrate histochemistry, positive PAS reaction indicates the presence of neutral carbonhydrate, while positive Alcian Blue reactions at pH 1.0 and 2.5 indicate the presence of acidic sulphated and acidic carboxylated residues respectively. Mucin synthesis and secretion are influenced by the diet [27]. However, in the present study, staining properties with PAS, Alcian blue (pH 1.0 and 2.5) and PAS/AB (pH 1.0 and 2.5) of goblet cells not showed marked differences between two groups. There was no effect of the SC on mucins, which secreted by goblet cells. This result may be due to the use of low dose of SC. In the present study, the Brunner's glands were stained strongly positive with PAS and PAS/AB pH 2.5 in the SC treatment group than those of the control group. But, they were stained sligthly with AB pH 1 in both diet groups. Our results showed that neutral and acidic mucins were enhanced by feed supplemented with SC in the Brunner's glands. The results suggest that SC have to be protecting against enteric pathogens. Ozpinar et al.[28] also reported, SC protects from invading pathogens by mucosal immunity.

In conclusion, the addition of SC to the diet of rabbits affected the morphology of the duodenum by increasing the total mucosa, villus height, and the gland depth with inducing enlargement of the Brunner's glands. However, the addition of SC also little affected the histochemical features of the duodenum by increasing the secretion of neutral and acidic mucins in the Brunner's glands. We think that the effects may be generally related to the dose of SC. It may be proposed that higher doses of yeast may be used for digestive health. In addition, further studies are necessary to obtain definitive evidence on the effects of yeast supplementation on digestive system.

## REFERENCES

**1. Hamilton SR, Bobrowicz P, Bobrowicz B, Davidson RC, Li H, Mitchell T, Nett JH, Rausch S, Stadheim TA, Wischnewski H, Wildt S, Gerngross TU:** Production of complex human glycoproteins in yeast. *Science*, 301, 1244-1246, 2003.

**2. Endo T, Nakano M, Shimizu S, Fukushima M, Miyoshi S:** Effect of a probiotic on the lipid metabolism of cocks fed on cholesterol-enriched diet. *Biotec Biochem*, 63, 1569-1575, 1999.

**3. Roberfroid M:** Prebiotics: The concept revisited. *J Nutr*, 137, 830, 2007.

**4. Saulnier DM:** Identification of prebiotic fructooligosaccharide metabolism in *Lactobacillus plantarum WCFS1* through microarrays. *Appl Environ Microbiol*, 73, 1753, 2007.

**5. Fuller R:** Probiotics in man and animals. *J Appl Bacter*, 66, 365-78, 1989.

**6. Jin LZ, Ho YV, Abdullah N, Jalaludin S:** Digestive bacterial enzyme activities in broilers fed diets suplemented with Lactobacillus cultures. *Poult Sci,* 79, 886-891, 2000.

**7. McFarland LV, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, Moyer KA, Greenwald DA:** Randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *JAMA*, 271, 1913-1918, 1994.

**8. Rodrigues ACP, Cara DC, Fretez SHGG, Cunha FQ, Vieria EC, Nicoli JR, Vieira LQ:** *Saccharomyces boulardii* stimulates sIgA production and the phagocytic system of gnotobiotic mice. *JAM*, 89, 404-414, 2000.

**9. Falcão-e-Cunha L, Castro-Solla L, Maertens L, Marounek M, Pinheiro V, Freire J, Mourão JL:** Alternatives to antibiotic growth promoters in rabbit feeding: A review. *World Rabbit Sci*, 15, 127-140, 2007.

**10. Sandıkcı M, Eren U, Onol AG, Kum S:** The effect of heat stress and the use of *Saccharomyces cerevisiae* or (and) bacitracin zinc against heat

stress on the intestinal mucosa in quails. *Rev Med Vet*, 11, 552-556, 2004.

**11. Pelicano ERL, Souza PA, Souza HBA, Figueiredo DF, Boiago MM, Carvalho SR, Bordon VF:** Intestinal mucosa development in broiler chickens fed natural growth promoters. *Rev Bras Cienc Avic*, 7, 221-229, 2005.

**12. McManus JFA:** Histological and histochemical uses of periodic acid. *Stain Technol,* 23, 99-108, 1948.

**13. Lev RA, Spicer SS:** Specific staining of sulphate groups with Alcian Blue at low pH. *J Histochem Cytochem*, 12, 309, 1964.

**14. Mowry RW:** The special value of methods that colour both acidic and vicinal hydroxyl groups in the histochemical study of mucins with revised directions for the colloidal iron stain, the use of Alcian blue 8GX, and their combination with the periodic acid-Schiff reaction. *Ann N Y Acad Sci*, 106, 402-423, 1963.

**15. Pluske JR, Thompson MJ, Atwood CS, Bird PH, Williams LH, Hartmenn PE:** Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. *Br J Nutr*, 76, 409-422, 1996.

**16. Buts JP, Keyser N, Raedemaker L:** *Saccharomyces boulardii* enhances rat intestinal enzyme expression by endoluminal release of polyamines. *Pediatr Res*, 36, 522-527, 1994.

**17. Baum B, Liebler-Tenorio EM, Enss ML, Pohlenz JF, Breves G:** *Saccharomyces boulardii* and *bacillus cereus var. Toyoi* influence the morphology and the mucins of the intestine of pigs. *Z. Gastroenterol,* 40, 277-284, 2002.

**18. Samanya M, Yamauchi KE:** Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. *natto. Comp Biochem Physiol A Mol Integr Physiol,* 133, 95-104, 2002.

**19. Santin E, Maiorka A, Macari M, Grecco M, Sanchez JC, Okada TM, Myasaka AM:** Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. *JAPR*, 10, 236-244, 2001.

**20. Bradley GL, Savage TF, Timm KI:** The effects of supplementing diets with *Saccharomyces cerevisiae* var. *boulardii* on male poult performance and ileal morphology. *Poult Sci*, 73, 1766-1770, 1994.

**21. Schumacher U, Duku M, Katoh M, Jorns J, Krause WJ:** Histochemical similarities of mucins produced by brunner's glands and pyloric glands: A comparative study. *Anat Rec A Discov Mol Cell Evol Biol,* 278, 540-550, 2004.

**22. Specian D, Oliver M:** Functional biology of intestinal goblet cells. *Am J Physiol*, 260, 183-193, 1991.

**23. Forstner JT:** Intestinal mucins in health and disease. *Digestion*, 17, 234-263, 1978.

**24. Crescenz A, Barsotti P, Anemona L, Marinozzi V:** Carbohydrate histochemistry of human Brunner's glands. *Histochem,* 90, 47-49, 1988.

**25. Takehana K, Eerdunchaoluo HU, Kobayashi A, Iwasa K, Sou K:** A histochemical study of the camel *(Camelus bactrianus)* duodenal glands. *J Vet Med Sci,* 62, 449-452, 2000.

**26. Krause WJ:** Brunner's glands: A structural, histochemical and pathological profile. *Prog Histochem Cytochem*, 35, 255-367, 2000.

**27. Sharma R, Schumacher U:** Morphometric analysis of intestinal mucins under different dietary conditions and gut flora in rats. *Dig Dis Sci,* 40, 2532–2539, 1995.

**28. Ozpinar H, Aydin IH, Klasing KC, Tekiner IH:** Interaction of Mannan oligosaccharide with immune system 'transport of MOS in to the lamina propria'. *Kafkas Univ Vet Fak Derg*, 18 (1): 121-128, 2012.