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Saccharomyces boulardii as an enteric health promoter in broiler chickens

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Saccharomyces boulardii as an enteric health promoter in broiler chickens

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Abstract

Saccharomyces boulardii is a yeast that has been shown to have a probiotic effect on the gut health of humans, ruminants, and swine by helping to control intestinal homeostasis, preventing pathogens from colonizing, promoting beneficial enzyme production, and by other mechanisms. This trial is designed to test whether the addition of *S. boulardii* to the diet improves the enteric health of broilers. Forty-eight test pens each containing fifteen birds, a water line, and a hanging feed pan were set up in a commercial broiler house from day eighteen to day thirty-six of the flock. The pens were evenly divided into six treatments: one control, and five different application methods and amounts of yeast. During the trial, weights, gains, and feed consumption were measured at 18, 28, and 36 days. After the trial concluded, samples of the duodenum and ileum were taken for determination of villi length and crypt depth and to test for bacitracin resistant strains of bacteria. The results showed that with an alpha of 0.05 *S. boulardii* had no significant impact on production characteristics at any concentration, and had a small but significant negative effect on gut health.

Introduction

Chickens raised for meat experience enteric challenges, often caused by an imbalance in the intestinal microflora. The effect of these challenges may be visible, such as diarrhea, or they may only be shown as high feed conversion or low body weights. One way to help prevent these problems is by introducing more beneficial bacteria (Kabir, 2009). Supplementing broilers' diets with *S. boulardii*, a yeast probiotic, may be one solution.

The objective of this research project was to determine whether *S. boulardii* is effective in improving the gut health of broilers, and if it was, to determine the best method and amount of

application. This was determined by evaluating bird weights, feed consumption, antibiotic-resistant bacteria count, and villi and crypt measurements. The objective of the current study was to determine a correlation between one or more of the *S. boulardii* treatments.

Literature Review

Bacteria begin colonizing chickens' intestines and cecae soon after hatch. The colonies begin to stabilize at around seven days post hatch (Lu, 2003). In the small intestine, comprised of the duodenum, ileum, and jejunum, the flora is a mix of anaerobes and aerobes. The microflora in the cecae is predominantly anaerobic. The types of microflora in the small intestine include *Streptococcus, Staphylococcus, Lactobacillus, and Escherichia coli*, all of which are facultative anaerobes; and *Eubacterium, Propionibacterium, Clostridium, Gemmiger, and Fusobacterium*, which are all strict anaerobes (Salanitro, 1977). There have been 117 established genera of bacteria identified in the intestines of chickens (Wei, 2012).

The microflora is important because of its effect on the immune system and its effect on metabolism. The lining of the intestines is the largest exposed area of a chicken's body. It is important for the health of the bird that the microflora be in an appropriate balance (Yegani, 2008). The gut-associated lymphoid tissue (GALT) performs some immune functions. Animals without microflora are more likely to have infections. Introducing microflora enables a humeral immune response, and also improves the cell mediated immune response (Cebra, 1999).

An imbalance of microflora usually increases the nutrient requirements of chickens (Furuse, 1984). However, if the diet is high in fiber or if the birds are in feed withdrawal, the microflora reduces the energy requirement (Muramatsu, 1994). Any microflora increases the

nutrient needs of broilers compared to broilers without microflora. However, commercial broilers cannot be raised without it, so it is best if the microflora is beneficial (Jin, 1996).

The types of microflora are controlled by what the birds are exposed to. One way to change what the birds are exposed to is through environmental management: Clean out and other sanitation methods are used to reduce exposure to pathogens (Hughes, 2005). However, these methods are not used to increase exposure to beneficial organisms. Another way to control or limit exposure is through feed additives, such as by using antibiotic growth promoters (AGPs), or by using probiotics (Yang, 2009).

Antibiotic growth promoters are low doses of antibiotics often added to feed by poultry integrators**.** One way AGPs work is by reducing the number of pathogens in the gut of the chicken so that energy is directed towards growth rather than towards coping with or fighting off illnesses by reducing nutrient destruction by bacteria (Butaye, 2003). Another way they may work is by acting as an anti-inflammatory. This reduces sub-clinical infection, could reduce products that slow metabolism, and increase absorption of nutrients (Niewold, 2007). Even though AGPs work, there has been recent pressure from consumers and the government to end the use of AGP. This will likely be a reality for the poultry industry in the near future, so the industry must be looking for alternatives (Dibner, 2005).

One alternative to AGPs is probiotics. Probiotics are becoming more common in poultry because of differing types of pathogens, development of resistance to AGPs, and consumer perceptions (Kabir, 2009). Probiotics are live microorganisms that are beneficial for an animal (Czerucka, 2007). They may include bacteria or yeast, and may work in different ways: First, by helping the immune system to fight infectious diseases or to reduce intestinal inflammation;

second, by directly affecting other bacteria, including pathogens that are in the gut; or, third, by deactivating toxins and detoxifying the gut (Succol, 2010).

S. boulardii, the probiotic being tested, is one supplement that may provide enteric benefits for broilers. It was originally isolated from the lychee plant from India and Southeast Asia. Locals had been using the fruit as a cure for diarrhea (Toma, 2005). Now one source of this yeast is in a product called Luvacell, produced by an animal health company called Lallemand. One study showed that supplementing broiler feed with yeast decreased feed consumption and improved feed efficiency, while also increasing villi size as compared with an AGP (Ghosh, 2012).

S. boulardii may work in several different ways: including helping to control intestinal homeostasis, by preventing pathogens from colonizing, by promoting beneficial enzyme production, by improving the gastrointestinal lining permeability, or by improving immune responses (Kelesidis, 2012). It has been shown to be effective in improving the health of both humans and swine (Rajowska, 2012; Schroeder, 2004).

Ways to measure the intestinal health of poultry include measuring the depths of crypts and the length of villi, and by testing bacitracin resistance. It can also be inferred from production characteristics such as feed consumption, feed efficiency, and weight gain.

Villi provide the intestine of the bird with a very large surface area to increase absorption. If the villi are longer the bird has more possible absorption, so it is an indicator of gut health (Awad, 2008). Because the crypts are where villi are formed, a shallower crypt is also an indicator of better gut health. If the crypt is very deep, it indicates that there is rapid turnover of villi (Choct, 2009).

Bacitracin is commonly added to poultry feed to control necrotic enteritis. Although this doesn't create microbial resistance to humans, poultry microbes can develop a resistance to it. Having more bacitracin resistant bacteria in the intestine increases the risk of necrotic enteritis and other illnesses (Phillips, 1999).

Materials and Methods

This project was designed to test the benefit of a yeast supplement consisting of *S. boulardii* in an alfalfa carrier, and to determine the most effective delivery method and dose. This research was conducted in two commercial broilers houses at the Savoy broiler research farm.

Houses 1 and 2 each had twenty-four test pens set up down the middle of the front half of each barn, as shown in figure 1. Each pen was 1.22 meters long by 1.22 meters wide and contained a water line with three nipples and a feeder, both adjustable and supported by a frame placed on top of the cage, as shown in figure 2. Every day the pens were checked to make sure that they were in good shape, the water line height was adjusted to the correct level, and the feeder was tested to ensure that they were not restricting consumption or causing feed wastage. Since the pens were made of wire and were placed in a typical, commercial broiler house being used for grow out, the birds were on commercial lighting and ventilation schedules. They were also exposed to the typical litter and air conditions, as well as typical socking densities. The stocking density was 0.1 square meters per bird, compared to the typical density of 0.074 square meters per bird. Part of this was due to the space that the feeder and water line took in the pen.

Each of the forty-eight pens had fifteen male Cobb 500 broilers placed on day eighteen. The weights were collected and recorded at day 18, 28, and 36. All the feed added was weighed,

as well as the feed not consumed. At day 18, 28, and 36, all of the feeders were weighed to allow for comparing average daily consumption.

The pens were randomly divided into six groups, each of which had a different treatment. Group 1 was the control, so it did not receive any yeast. Groups 2 through 5 had the supplement top dressed on the feed, according to the schedule in Table 1, below. The amount used was calculated by using the desired rate, multiplied by the amount of feed the chickens were expected to eat at the specific day of age. The amount the birds were expected to eat was calculated from the Cobb 500 grower guide and is shown in Table 2, below. Group 6 received the supplement mixed in a small cement mixer with the feed at a rate of 1lb. supplement per ton of feed. The feed used was commercial grower and finisher feed.

Table 1: Treatments

	Day 18-22	Day 21-Completion	Presentation
Treatment 1 (Control)	0	0	
Treatment 2	2 lb. per ton	1 lb. per ton every day	Top dressed
Treatment 3	2 lb. per ton	½ lb. per ton every day	Top dressed
Treatment 4	2 lb. per ton	1 lb. per ton every other day	Top dressed
Treatment 5	2 lb. per ton	None	Top dressed
Treatment 6	1 lb. per ton	1 lb. per ton	Mixed in

Table 2: Expected consumption, from Cobb 500 grower manual

Day of Age	18	19	20	21	22	23	24
Daily Consumption per bird	0.201	0.214	0.227	0.24	0.258	0.271	0.293
Consumption for 15 birds (lbs)	3.02	3.21	3.41	3.60	3.87	4.07	4.40

Day of Age	25	26	27	28	29	30	31
Daily Consumption per bird	0.311	0.326	0.342	0.357	0.375	0.392	0.406
Consumption for 15 birds (lbs)	4.67	4.89	5.13	5.36	5.63	5.88	6.09

Day of Age	32	33	34	35	36	37	38
Daily Consumption per bird	0.428	0.443	0.459	0.474	0.478	0.483	0.487
Consumption for 15 birds (lbs)	6.42	6.65	6.89	7.11	7.17	7.25	7.31

At the conclusion of the trial, the birds were all weighed by pen, and the remaining feed was weighed. One bird per pen was weighed and then sacrificed via cervical dislocation, a method approved by the AVMA. Sections of the duodenum and ileum were collected and placed in formalin. After fixation, cross sections were fixed onto microscope slides and were measured for villi height and crypt depth using Image Pro Plus software to measure microscopically (Petersen, 2001). Ten villi and ten crypts were measured from each of the duodenum and ileum sections, as shown in figures 3 and 4. A second two inch section of the mid gut was sealed closed on both ends using sterile ties, cut loose from the remaining gut, and placed in sterile bags for determination of Bacitracin resistant clostridium. The data generated from the production and from the samples was used for determination of any benefit based on weight gain, feed conversion, or gut health.

All of the statistical results were calculated using an ANOVA test in Excel with an alpha of 0.05. The ANOVA test tests the variance within groups and between groups to determine if

there was a statistical difference. The alpha of 0.05 indicates that there is a 95% chance that a statistical difference is due to factors other than chance.

Results

Weights

The average weights for each treatment are summarized in Table 3. If there was any mortality, the weight of any mortality was added to the group pen weight for the calculation of an adjusted pen weight. On day 18, when the trial began, there was no significant difference in the weights and the variance was very small, indicating that all the treatments started with similar bird weights. On days 28 and 36, the weight difference was also insignificant. The difference in weight gain from both 18-28 days and 28-36 days was also insignificant, with P-values of 0.8913 and 0.1200, respectively (data not shown).

Table 3: The effect of feeding broilers *S. Boulardii* Average weight of broilers in kilograms

	18 days		28 days		36 days	
Treatment	*Average*		*Average*		*Average*	
1 (Control)	0.755	± 0.009	1.605	± 0.022	2.341	± 0.022
2 (1 lb. ED, TD)	0.752	± 0.006	1.632	± 0.006	2.341	± 0.006
3 (1/2 lb. ED, TD)	0.747	± 0.005	1.595	± 0.033	2.275	± 0.033
4 (1 lb. EOD, TD)	0.748	± 0.008	1.593	± 0.031	2.290	± 0.031
5 (none)	0.752	± 0.011	1.614	± 0.017	2.274	± 0.017
6 (1lb. ED, MI)	0.757	± 0.008	1.581	± 0.027	2.254	± 0.027
P-value		**0.9402**		**0.7437**		**0.3105**

The notation next to treatment number indicates the treatment method and amount from day 21 through completion. ED = every day, EOD = every other day, TD = top dressed, and MI = mixed in.

Feed Intake

The feed intake and feed conversion are shown in Table 4, below. There were no significant differences related to feed usage, as the P-values ranged from 0.2604-0.4673. Group 1, the control group, did have a lower feed conversion than the other groups, indicating better feed efficiency, but the difference was not significant.

Table 4: Effect of feeding broilers *S. Boulardii* on feed intake in kg per bird and feed conversion

	Feed Intake period				Feed Conversion period			
	18-28		28-36		18-28		28-36	
Treatment	*Average*		*Average*		*Average*		*Average*	
1 (Control)	1.39	± 0.02	1.51	± 0.04	1.64	± 0.04	2.07	± 0.08
2 (1 lb. ED, TD)	1.40	± 0.01	1.55	± 0.05	1.60	± 0.01	2.30	± 0.13
3 (1/2 lb. ED, TD)	1.35	± 0.03	1.50	± 0.05	1.61	± 0.03	2.39	± 0.12
4 (1 lb. EOD, TD)	1.36	± 0.04	1.55	± 0.04	1.62	± 0.04	2.36	± 0.08
5 (none)	1.34	± 0.02	1.45	± 0.09	1.56	± 0.03	2.23	± 0.07
6 (1lb. ED, MI)	1.38	± 0.02	1.62	± 0.06	1.69	± 0.04	2.57	± 0.08
P-value	**0.4673**		**0.4071**		**0.1637**		**0.2390**	

Bacitracin Resistance

The results of the bacitracin resistance test are shown in table 5, below. All of the samples that tested positive for anaerobic cultures only had one type of bacitracin resistant bacteria present, except for treatment 1. One sample in treatment 1 had both *Clostridium clostridiforme* and *Bifidiobacterium* that were resistant.

Table 5: Effect of feeding broilers *S. Boulardii* on presence of bacitracin resistant bacteria

Treatment	Strains	% birds testing positive
1 (Control)	*Clostridium tertium* *Clostridium sporengenes* *Clostridium clostridioforme* *Bifidiobacterium*	37.5
2 (1 lb. ED, TD)	*Clostridium difficile* *Clostridium sporengenes*	25.0
3 (1/2 lb. ED, TD)	*Clostridium sporengenes*	12.5
4 (1 lb. EOD, TD)	*Clostridium bifernentans*	12.5
5 (none)	*Collinsella aerofaciens* *Clostridium perfringens*	25.0
6 (1lb. ED, MI)	*Propionibacterium acnes* *Collinsella aerofaciens* *Clostridium perfringens*	37.5

Gut Health

The results of the microscopic analysis of villi length and crypt depth are shown in Table 6 and Table 7, below, for the duodenum and ileum, respectively.

In the duodenum, the villi length had a P-value of 0.0982, and was not significantly different with an alpha of 0.05. However, the control group had the shortest villi.

The duodenal crypt depth was significantly different, with a P-value of 0.0173. The significant difference was between treatment 5, the group that received the supplement top dressed at a rate of 2 lbs. per ton of feed for the first three days and then none after that, and between treatment 1, the control group. Treatment 5 had the deepest crypts, indicating the poorest health.

Table 6: Effect of feeding broilers *S. Boulardii* on duodenal crypt and villi health

	Duodenum							
	Villi Length			Crypt Depth			Heigth/Depth ratio	
Treatment	*Average*			*Average*			Average	
1 (Control)	3377.69	±76		837.32	±33	b	4.30	± 0.39
2 (1 lb. ED, TD)	3535.39	±71		931.5	±33	ab	3.92	± 0.32
3 (1/2 lb. ED, TD)	3670.41	±71		933.12	±33	ab	4.15	± 0.45
4 (1 lb. EOD, TD)	3629.63	±73		857.51	±33	ab	4.39	± 0.29
5 (none)	3536.03	±71		986.47	±33	a	3.82	± 0.42
6 (1lb. ED, MI)	3591.26	±72		890.94	±33	ab	4.09	± 0.31
P-value	**0.0982**			**0.0173**			**0.8839**	

Both the villi length and the crypt depth of the ileum were significant. The control treatment had the shortest villi but also the most shallow crypts, even though the difference was not always significant. The P-value for the villi was 0.0101, with the difference occurring between treatment 4, which received the supplement top dressed at 2 lbs. per ton the first three days and 1 lb. per ton until the conclusion, and the control group.

The crypts in the ileum were also significantly different, with a P-value of 0.0001. The treatments which were significantly different are noted in Table 7 by the letters. The only treatment which was not significantly different from the control was treatment 4.

Table 7: Effect of feeding broilers *S. Boulardii* on ileum crypt and villi health

	Ileum							
	Villi Length			Crypt Depth			Heigth/Depth ratio	
Treatment	*Average*			*Average*			*Average*	
1 (Control)	1892.07	±86	b	413.87	±59	c	5.08	± 0.63
2 (1 lb. ED, TD)	2177.62	±64	ab	528.58	±36	ab	4.04	± 0.21
3 (1/2 lb. ED, TD)	2119.32	±75	ab	573.96	±64	a	3.79	± 0.24
4 (1 lb. EOD, TD)	2310.11	±69	a	464.94	±77	bc	4.99	± 0.49
5 (none)	2176.72	±61	ab	611.62	±34	a	3.62	± 0.20
6 (1lb. ED, MI)	2096.63	±61	ab	534.40	±50	ab	4.04	± 0.24
P-value	**0.0101**			**0.0001**			**0.0622**	

Livability

There was no significant difference in the livability of the birds between treatments, as shown in Table 8. However, the control treatment had the lowest mortality, followed by the treatment that only received the supplement for five days.

Table 8: Effect of feeding broilers *S. Boulardii* on livability

Treatment	Mortality	Livability
1 (Control)	0	100.00%
2 (1 lb. ED, TD)	3	97.50%
3 (1/2 lb. ED, TD)	2	98.33%
4 (1 lb. EOD, TD)	2	98.33%
5 (none)	1	99.17%
6 (1lb. ED, MI)	5	95.83%
P-value	**0.22525**	

Discussion

This trial showed that the addition of the *Saccharomyces boulardii* supplement did not affect the production characteristics of weight, weight gain, or feed consumption, and actually may have slightly impaired the feed efficiency, although not at a significant level. This indicates that the supplement is ineffective at improving the production measures of a healthy flock.

The gut health of the birds receiving the supplement was significantly affected. The control group had the highest count of bacitracin resistant bacteria at 4, compared to 0-3 counts in the other treatments. Treatment 4 had significantly deeper crypt depths than the control, but also had significantly longer ileal villi. All of the treatments except treatment 4 had significantly deeper crypt depths than the control, indicating a negative effect by the treatments.

This trial was performed on a healthy flock that was not challenged, and was also already receiving Narasin, Nicrabazin, and Basitracin methylene disalicylate in the commercial grower feed. If the broilers had been challenged or had not been supplemented with AGPs, the results

could have potentially presented a greater change in gut health, which could in turn improve the production measures. More research would have to be done to provide conclusive evidence on whether *Sacccharomyces boulardii* would be effective in treating challenged flocks.

Another factor likely affecting the results of this trial was the start date of the trial, at 18 days. As the microflora of the gut is relatively established by seven days of age, eighteen days may have been too late to try to manipulate the flora with the addition of a probiotic. The results may have been different if this research were done on younger birds.

The data from this trial suggests that *Saccharomyces boulardii* is not effective in significantly improving the performance of healthy broilers, but instead could actually be detrimental to their health.

Appendix 1: Works Cited

Awad, Ghareeb, Bohm. 2008. Intestinal Structure and Function of Broiler Chickens on Diets Supplemented with a Synbiotic Containing Enterococcus faecium and Oligosaccharides. International Journal of Molecular Science. 9(11), Pgs. 2205-2216.

Butaye, Patrick. "Antimicrobial Growth Promoters Used in Animal Feed: Effects of Less Well Known Antibiotics on Gram-Positive Bacteria." *Clinical Microbiology Review* (2003): 175-188.

Cebra. 1999. Influences of microbiota on intestinal immune system development. *American Journal of Clinical Nutrition* 69: 1046–1051.

Choct. 2009. Managing gut health through nutrition. *British Poultry Science*. 50(1). Pgs. 9-15.

Czerucka, D. "Yeast as Probiotics – Saccharomyces boulardii." *Alimentary Pharmacology & Therapeutics* (2007): 767-778.

Dibner, J.J. (2005). Antibiotic Growth Promoters in Agriculture: History and Mode of Action. *Poultry Science.* 634-643.

Furuse, M. & Yokota, H. (1984). Protein and energy utilization in germ-free and conventional chicks given diets containing different levels of dietary protein. *British Journal of Nutrition.* 51, 255-264.

Ghosh, T.K. (2012). Assessment of yeast cell wall as replacements for antibiotic growth promoters in broiler diets: effects on performance, intestinal histo-morphology and humoral immune responses. *Journal of Animal Physiology and Animal Nutrition*. 275-284.

Hughes. 2005. An integrated approach to understanding gut health and gut function of chickens. *Asia Pacific Journal of Clinical Nutrition.* 14(suppl.)

Jin, L., Ho, Y., Abdullah, N., Jalaludin, S. (1996). Influence of dried *Bacillus subtilis* and lactobacilli cultures on intestinal microflora and performance in broilers. *Asian-Australian Journal of Animal Sciences*. 9(4), pgs 397-403.

Kabir. 2009. The Role of Probiotics in the Poultry Industry. *International Journal of Molecular Science.* 10(8): 3531–3546.

Kelesedis. 2012. Efficacy and safety of the probiotic *Saccharomyces boulardii* for the prevention and therapy of gastrointestinal disorders. *Therapeutic Advanced Gastroenterology*. 5(2): 111–125.

Lu, Idris, Harmon, et al. 2003. Diversity and Succession of the Intestinal Bacterial Community of the Maturing Broiler Chicken. *Applied and Environmental Microbiology*. 81(7).

Muramatsu, Nakajima, Okumura. 1994. Modification of energy metabolism by the presence of the gut microflora in the chicken. *British Journal of Nutrition*. 71. Pgs. 709-717.

Niewold. 2007. The Nonantibiotic Anti-Inflammatory Effect of Antimicrobial Growth Promoters, the Real Mode of Action? A Hypothesis. *Poultry Science*. 86 (4). Pgs. 605-609.

Petersen, Y., Burrin, D., Sangild, P. (2001). GLP-2 has differential effects on small intestine growth and function in fetal and neonatal pigs. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*. 281(6). Pgs. 1986-93.

Phillips. 1999. The use of bacitracin as a growth promoter in animals produces no risk to human health. *Journal of Antimicrobial Chemotherapy*. 44 (6). Pgs. 725-728.

Rajkowska, Katarzyna. "Probiotic Activity of Saccharomyces cerevisiae var. boulardii Against Human Pathogens." *Food Technology and Biotechnology* (2012): 230-236.

Salanitro, Blake, Muirhead, et al. 1977. Bacteria Isolated from the Duodenum, Ileum, and Cecum of Young Chicks. *Applied and Environmental Microbiology*. 35(4). Pg. 782-790.

Schroeder, Winkler, Failing, et al. 2004. Studies on the time course of the effects of the probiotic yeast Saccharomyces boulardii on electrolyte transport in pig jejunum. *Digestive Diseases and Sciences.* 49(7-8):1311-7.

Soccol, C.R. "The Potential of Probiotics: A Review." *Food Technology and Biotechnology* (2010): 413-433.

Toma, Raipulus, Kalnina, et al. 2005. Effect of Probiotic Yeast on Genotoxicity: Does Probiotic Yeast Act as Antigenotoxin? *Food Technology and Biotechnology*. 43 (3). Pgs. 301–305.

Wei, Morrison, Yu. 2012. Bacterial census of poultry intestinal microbiome. *Poultry Science* (2013) 92 (3):671-683.

Yang, Choct. 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poultry Science Journal*. 65(97-113).

Yegani, Korver. 2008. Factors Affecting Intestinal Health in Poultry. *Poultry Science*. 87(10). Pg. 2052-2063.

Appendix 2: Figures

Figure 1: The setup of the barn

Figure 2: The setup of the pens

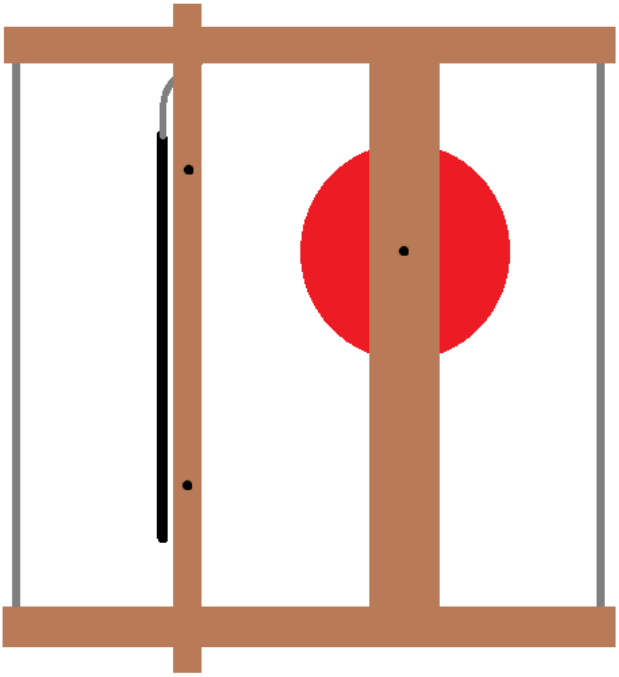

Figure 3: Microscopic view of duodenal villi, showing how villi length and crypt depth were measured

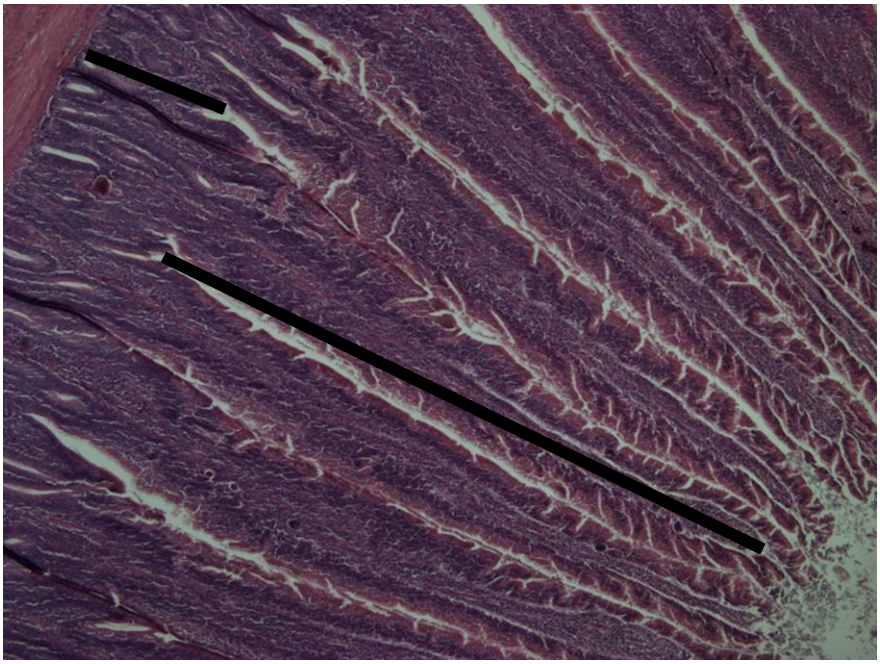

Figure 4: Microscopic view of ilial villi, showing how villi length and crypt depth were measured