Reduction of food phatogens prevalence in dietary *S. cerevisiae*-fed poultry orally challenged with *S. enteritidis* and *C. jejuni*

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The effect of yeast supplementation in broiler chickens on *Salmonella enteritidis* and *Campylobacter jejuni* contamination in faeces, cecum, breast, and neck skin was evaluated. Two groups (12 replicates/group, 20 Hubbard female chickens 1d old/replicate) were fed pre-starter (0-10d), starter (11-20d) and growing (21-38d) diets, and administered (Y) or not (C) Levucell® SB20 (*Saccharomyces cerevisiae* type *boulardii* I-1079; 10^6 CFU/g feed through a 0.05% premix). Birds were orally challenged at 10 days of life (*S. enteritidis*, $1x10^5$ CFU/bird, and *C. jejuni,* $3x10^5$ CFU/bird). On day 10 and 28 post-infection (PI), 10 animals/replicate were slaughtered and pooled ceca content of 5 birds/replicate was analysed for *Salmonella* and *Campylobacter* detection and enumeration together with total yeast count. Neck and breast skin were tested for *Salmonella* and *Campylobacter* presence on 1 subject/replicate. Data were analysed by a GLM procedure of SAS considering two experimental periods, from 0 to 20 days and from 20 to 38 days. Growth performance and faecal coliforms content were not affected by treatment. Higher yeast and lactobacilli (P=0.01) faecal count, and a significant decreased *Salmonella* enumeration and frequency in neck (-41%, P=0.03) and tendency in faeces (-25%; P=0.06), cecum (-25%; P=0.06), and breast skin (-33%; P=0.08) were found in Y group on day 38. No fecal *Campylobacter* was detected at 10d (P<0.01) or 28d (P=0.06) PI in Y birds, while in neck skin absence of *Campylobacter* was only recorded on day 10 PI (P=0.01). *Campylobacter* was significantly lower in Y birds in cecum (-42%; P=0.01), and breast skin (-58%; P=0.04) on 10d PI, while on day 38 it was reduced in breast skin (-42%; P=0.02), and tended to decrease in faeces (-25%; P=0.06). *Saccharomyces cerevisiae* (CNCM I-1079) significantly controlled *Campylobacter* carriage in chickens with some positive results also on *Salmonella* contamination, thus reducing the contamination of carcasses with both food borne pathogens.

Keywords: *Saccharomyces boulardii*; *Salmonella*; *Campylobacter*; live yeast; chicken, food safety

Introduction

During past years probiotics has been studied in different animal species (Quarantelli et al., 2008; Ripamonti et al., 2009, 2011; Agazzi et al., 2011) with positive results on performance and health status. These kind of compounds have been shown to be involved in protection against a variety of pathogens in

chicken including *Escherichia coli* (Chateau et al., 1993), *Salmonella* and *Campylobacter* (Stern et al., 2001), *Clostridium* and *Eimeria* (Dalloul et al., 2005). The mechanisms of action in poultry is based on positive alterations in intestinal microflora population by competitive exclusion, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients (Yeo et al., 1997). In addition probiotics have shown also to interact with the host by influencing the immune response (Delcenserie et al., 2008), or producing components able to positively affect mucosa development or the metabolism of the host's intestinal cells (Johnson-Henry et al., 2008). Therefore, the major outcomes using probiotics include improvement in growth (Yeo et al., 1997), reduction in mortality (Kumprecht, 1998), and improvement in feed conversion rate (Kizerwetter-Swida, et al., 2009). The aim of this trial was to investigate the effects of live yeast supplementation on *Salmonella* and *Campylobacter* contamination of the end product.

Materials and methods

The trial was performed at the Teaching and Research Zootechnical Centre of the Faculty of Veterinary Medicine of the Università degli Studi di Milano, Lodi, Italy. Four hundred eighty 1 day-old Hubbard female chickens were randomly allotted to one of the 2 experimental groups on the basis of dietary treatment for a period of 38 days. Each experimental group had 12 replicates (5x3 mt^2) of 20 subjects each. Both groups were allocated in the same room with controlled environmental conditions and free access to water. Initial room climate program considered a temperature equal to 33°C under the brooder and 30°C in the living area with a humidity of 60%, and a ventilation of $1m^3$/kg body weight (BW) until day 21. From day 22 of age until 35 the experimental room had 26°C under the brooder, 23 in the living area, 65% humidity and a ventilation of $3.4m^3$/kg BW. In the last three days of the trial room temperature was maintained at 19°C with 65% humidity and a ventilation of $3.4m^3$/kg BW.

Experimental groups consisted of: C) fed a basal diet; Y) fed the same basal diet as C with the supplementation of *S. cerevisiae boulardii* strain I-1079 (Levucell® SB20, , Lallemand SAS, France) at a concentration of $1x10^6$ CFU/g feed. A prestarter (0-10d), a starter (11-20d) and a grower (21-38d) basal diet were adopted during the trial with decreasing crude protein (CP: from 23.40% to 17.90% as fed) and increasing ether extract (EE: from 6.60% to 8.20% as fed) content. On day ten from the arrival, all experimental chickens were challenged by oral gavage (Line *et al.* 1998) with *S. enteriditis* ($1x10^5$ cfu/bird), and *C. jejuni* ($3x10^5$ cfu/bird).

Individual BW and feed intake (FI) per pen were recorded on day 0, 10, 20 and 38, thus average daily gain (ADG) and feed conversion rate (FCR) per pen were calculated. Pooled pen fresh droppings (20 grams) were collected at the end of each feeding phase for quantification of total lactic acid bacteria (ISO 16140:2003), total coliforms (ISO 16140:2003) and yeast (ISO 21527-1:2008) and the detection and the enumeration of *Salmonella* (ISO 6579:2002/Amendment 1:2007 protocol) and *Campylobacter* (Wagenaar, 2012). At 10 days post infection (PI), 10 animals per replicate were slaughtered and pooled ceca contents were analyzed for *Salmonella* and *Campylobacter* detection and enumeration. Moreover cecum content was analyzed for total yeast count, while neck and breast skin presence of *Salmonella* and *Campylobacter* was determined on one chicken per pen. At the end of the trial, all the remaining chickens were slaughtered and analyzed as described for day 10 PI.

Data on growing performance were split according to the day of challenge in two separate periods from 0 to 20 and from 21 to 38 days. Body weight, ADG, FI and FCR for 0-10 and 0-20 d were analyzed applying a Mixed procedure of SAS package (SAS/STAT, Version V8, SAS Inst., Inc., NC, USA, 2006), while 0-20 and 0-38d were analyzed by a GLM procedure in a randomized block design. A MIXED procedure was applied for microbiological parameters. The models included the treatment as fixed effect and the replicate was considered the experimental unit.

Results and discussion

In the present study the positive effects of yeast administration seems to be linked with the microbial carriage reduction that could lead to a beneficial impact on food safety rather than increased performance. Our data show that dietary treatment did not have any significant effect on growth (*Table 1*), confirming findings of some other authors (Al-Zenki et al., 2009).

Table 1: Growth performance of chicken broilers fed S. cerevisiae (1 x 10^6 CFU/g of feed) and orally challenged with *S. entereditis* (1x10^5 cfu/bird), and *C. jejuni* (3x10^5 cfu/bird) at ten days of life (^{A,B} p<0.01).

	Days	Animals/replicate (n.)	Group		SEM	P		
Item			**C**	**Y**		**Treat.**	**Time**	**Treat x Time**
BW(g/head)	0	20	40.56	40.92	4.70	<0.01	<0.01	0.03
	10	20	254.45	246.13				
	20	20	728.15^{A}	710.13^{B}				
	21	10	730.37	705.54	35.44	0.82	<0.01	0.46
	38	10	1960.28	1973.44				
ADG (g/head/day)	0-10	20	21.39	20.52	0.51	<0.01	<0.01	0.89
	10-20	20	47.37	46.40				
	0-20	20	34.38	33.46	0.29	0.38	--	--
	21-38	10	68.33	70.44	1.49	0.35	--	--
FI (g/head/day)	0-10	20	26.90	27.07	0.81	0.43	<0.01	0.27
	10-20	20	72.40	71.29				
	0-20	20	49.64	49.18	0.64	0.48	--	--
	21-38	10	123.56	125.68	3.6	0.56	--	--
FCR	0-10	20	1.26^{B}	1.32^{A}	0.01	<0.01	<0.01	<0.01
	10-20	20	1.53	1.53				
	0-20	20	1.50	1.48	0.03	0.72	--	--
	21-38	10	1.81	1.78	0.02	0.13	--	--

On the contrary obtained positive results with increased fecal mean lactobacilli content in Y group than C (7.93 $\log_{10}$CFU/g vs 7.56 $\log_{10}$CFU/g; p<0.01) and decreased pathogen enumeration and count in fecal samples (*Table 2*) confirm that dietary live yeast administration in poultry can influence the microbial population of the gut being involved in the protection against a variety of pathogens including *Escherichia coli* (Chateau et al., 1993), *Salmonella* and *Campylobacter* (Line et al., 1998; Stern et al., 2001).

Coliforms presence in feces, although not statistically significant, was found to be lower in yeast-fed group, this result agrees with the findings of Chateau et al. (1993). The protection of the gut microflora is an important element in the health status of the host that ensures the local immunity, and its balance depends on the contact with environmental antigens, as competitive exclusion products, probiotics, or other immunostimulants that can contribute to immune stimulation and/or exclusion and prevention of pathogen colonization. The enhancement of colonization resistance and/or indirect inhibitory effects against pathogens are important factors where probiotics compounds could be effective in reducing the incidence and duration of diseases. The competition in attachment sites and/or for nutrients between yeast and pathogen microorganisms are popular hypothesis to explain the action of probiotics (Leser et al., 2008).

Table 2: Presence of yeasts, lactobacilli, and coliforms in feces of chicken broilers fed *S. cerevisiae* (1 x 10^6 CFU/g of feed) and orally challenged with *S. entereditis* (1x10^5 cfu/bird), and *C. jejuni* (3x10^5 cfu/bird) at ten days of life (^{A,B} p<0.01; ^{a,b} p<0.05).

	Day	Group		SEM	P		
Item		**C**	**Y**		**Treat.**	**Time**	**Treat x Time**
Coliforms (Log_{10}CFU/g)	0	11.47	11.79	0.48	0.10	<0.01	0.01
	10	8.37^{A}	6.60^{B}				
	20	8.31	8.25				
	38	7.14	6.99				
	0-38	8.82	8.41				
Yeasts (Log_{10}CFU/g)	0	0.39	0.30	0.17	<0.01	<0.01	<0.01
	10	0.43	0.76				
	20	1.01^{B}	2.26^{A}				
	38	1.24^{B}	3.80^{A}				
	0-38	0.77^{B}	1.79^{A}				
Lactobacilli (Log_{10}CFU/g)	0	7.40^{b}	8.00^{a}	0.24	<0.01	0.87	0.76
	10	7.59	7.89				
	20	7.66	8.00				
	38	7.58	7.84				
	0-38	7.56^{B}	7.93^{A}				

Many authors proposed that competitive exclusion could be used as a method to prevent *Salmonella* infection; numerous researchers have reported the ability of probiotic organisms to also reduce colonization of opportunistic pathogens in the gastrointestinal tract (Stern et al., 2001; Al-Zenki et al., 2009). In our study the frequency of *Salmonella* colonization was reduced in yeast-fed chickens in neck skin (-41%; *p=0.03*), and tended to decrease in feces (-25%; *p=0.06*), cecum (-25%; *p=0.06*), and breast (-33%; *p=0.08*) at the end of the trial (*Figure 1*), but the mean log numbers of *Salmonella* in feces, cecum, neck skin and breast skin was unaffected by yeast treatment differently from previous studies (Line et al., 1998, Al-Zenki et al., 2009). However, Line et al. (1998) similarly reported a reduction of *Salmonella* colonization in yeast-treated birds compared with the positive control. In our study the reduction of *Salmonella* frequency but not enumeration could outline a positive effect of yeast inclusion in the diet not on severity of contamination, but on the reduction of the total number of animals affected.

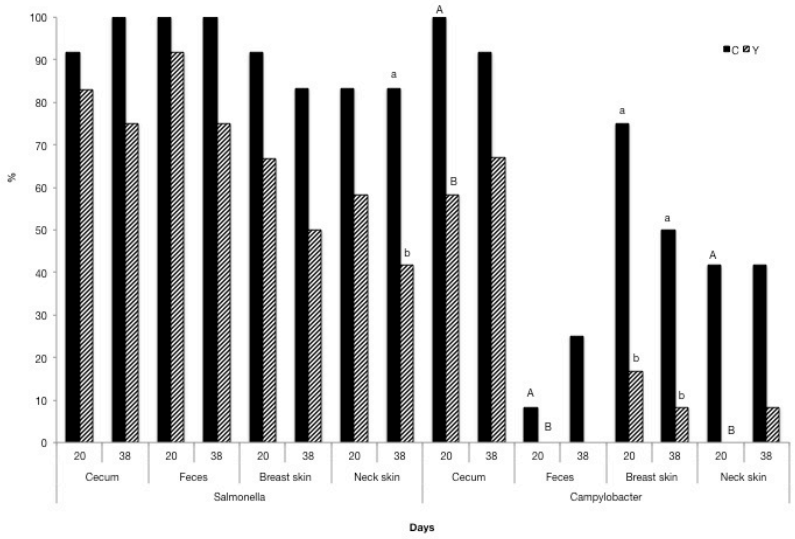

Figure 1 Frequency of *Salmonella* and *Campylobacter* in cecum, feces, breast skin and neck skin in chicken broilers fed *S. cerevisiae* (1 x 10^6 CFU/g of feed) and orally challenged with *S. entereditis* (1x10^5 cfu/bird), and *C. jejuni* (3x10^5 cfu/bird) on ten days of life (^{A,B} p<0.01; ^{a,b} p<0.05).

Campylobacter is one of the most common bacterial causes of foodborne illness, and a few studies have shown that probiotics may be able to reduce the amount of bacteria in chickens (Stern et al., 2001). In the present study, *Campylobacter* colonization was significantly lower in yeast-fed birds at first slaughtering time in cecum (-42%; *p*=0.01), and breast skin (-58%; *p*=0.04). The presence in feces (*p*=0.01) and neck skin (*p*=0.01) was not detected on day 10 PI in Y as opposed to C birds that were *Campylobacter* positive. At the second slaughtering time, *Campylobacter* in Y group tended to decrease in feces (-25%; p=0.06), cecum (-25%; p=0.13) and neck skin (-33%; p=0.06), while it was significantly reduced in breast skin (-42%; p=0.02) confirming the positive effect of yeast inclusion in poultry diet over these food pathogen bacteria.

Conclusions

The results of this study showed that feeding live yeast to chickens challenged with food pathogens microorganisms is able to reduce the frequency of pathogens such as *Salmonella* and *Campylobacter* in feces and on body surface. Controlling *Campylobacter* carriage and *Salmonella* contamination in chickens could lead to a reduced contamination of carcasses with both foodborne pathogens, resulting in safer foods for consumers.

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