Effect of dietary mannan oligosaccharide from *Saccharomyces cerevisiae* on live performance of broilers under *Clostridium perfringens* challenge

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Abstract

A 30-day broiler cage trial was conducted to evaluate the effect of dietary mannan oligosaccharide (MOS) from one commercial product (SAF-Mannan) on growth parameters, gut health and control pathogen colonization of broilers under *Clostridium perfringens* (*C. perfringens*) challenge. One hundred, 0-day-old male Ross 308 broilers were allocated in 4 experimental treatments for 30 days. The four dietary treatments were T1, standard broiler basal diets without any medication as a control (+CONT); T2, basal diets as in T1 plus *C. perfringens* challenge (-CONT); T3, enramycin 0.1 g/kg of feed plus *C. perfringens* challenge (ENRA); T4, SAF-Mannan at 0.5 g/kg in starter and finisher diets plus *C. perfringens* challenge (SAF). Overall, feed conversion ratio (FCR) and body weight gain (BWG) in treatments ENRA and SAF were significantly better (P<0.01) than the -CONT treatment, whereas treatment +CONT was intermediate and not different from SAF. Feed intake (FI) was not influenced by treatment. SAF-Mannan supplementation was able to lower the ileal *C. perfringens* count as compared to all other treatments (P<0.05). The changes in *C. perfringens* count appear in parallel to observed improvement in the cumulative FCR. The results from this study clearly indicated that SAF-Mannan could act as a replacement for antimicrobial growth promoters in broilers (AGPs). SAF-Mannan level of 0.05% was enough to achieve a response competitive with that of the antibiotic.

Introduction

There are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistances in pathogenic bacteria in both humans and poultry linked to the therapeutic and subtherapeutic use of antibiotics in livestock (Castanon, 2007). Current trends in poultry production point to reduction or total elimination of antimicrobial growth promoters (AGPs) use and increase the use of non-antibiotic feed additives that offer similar benefits, such as to improve the growth of broilers and improve the utilization of feed (Mountzouris et al., 2007). Several groups of these additives are in use such as probiotics, prebiotics, acidifiers, antioxidans and phyto-gene additives.

Prebiotics are a possible alternative to antibiotics in poultry diets. Prebiotic usually refers to oligosaccharides which are not digested by the animal enzymes, but can selectively stimulate certain intestinal bacteria species, which have potential beneficial effects on the host health. While probiotics are meant to bring beneficial microbes to the gut, oligosaccharides are supposed to selectively stimulate the beneficial microbes that already live there (Yang et al., 2009). Prebiotic have two advantages relative to probiotics: a technological, because there are no problems with the thermal processing of the feed and the acidic conditions of the digestive system, and a safety, because there is no introduction of any foreign microbial species into the gut. However, similar to probiotics, results of the effects of prebiotics on broiler performance are contradictory.

Mannan oligosaccharide (MOS) is derived from the outer layer of yeast cell walls, *Saccharomyces cerevisiae*. The effects of MOS on poultry production can be expressed in reduction of diseases by inhibition of pathogenic bacterial colonization to gut lining by binding to them and thus preventing them of proliferating and producing toxins (Benites et al., 2008), reducing intestinal pathogen counts (Benites et al., 2008), improving the immune system (Ferket, 2002) and exhibit influence on morpho-functional characteristics of intestines (Ferket, 2002; Zhang et al., 2005; Podmaniczky et al., 2006). These effects lead to better growth of broilers (Blake et al., 2006), improvement of FCR (Podmaniczky et al., 2006; Rosen, 2007). However, results of the effects of MOS on broiler performance are contradictory. Other reports showed that MOS had no positive influence on the performance of poultry (Waldroup et al., 2003).

There are limited reports on the effect of MOS on broilers under bacterial challenge. The objective of this study was to further determine the effects of MOS supplementation from SAF-Mannan® (S.I. LeSaffre, Marcq en Baroeul, France) to broiler diets compared to a growth promoting antibiotic (enramycin) on growth performance, histomorphology and bacterial count of small intestinal mucosa in broilers raised in cages under subclinical *C. perfringens* model and to determine the product with the most return and pathogen colonization control.

Materials and methods

Animals, husbandry and treatments

A total of 100, 0-day-old Ross 308 male broiler chicks obtained from a commercial hatchery (Al-Wadi Poultry Farm Co., Riyadh, Saudi Arabia) were placed in 20 cages (50 cm length, 60 cm width and 36 cm depth) in a four-deck cage system and received the experimental diets in electrically heated battery brooders with raised wire floors. The chicks had been vaccinated for Marek’s disease, Newcastle and infectious bronchitis. Birds were maintained...
at 23 h light schedule. The four dietary treatments were T1, standard broiler basal diets without any medication as a control (+CONT); T2, basal diets as in T1 plus C. perfringens challenge (-CONT); T3, 0.1 g/kg enramycin plus C. perfringens challenge (ENRA); T4, basal diets plus 0.5 g/kg SAF-Mannan plus C. perfringens challenge (SAF). On d 16, birds in treatments 2 to 4 were challenged by C. perfringens using overdose of anticyclicic vaccine namely Paraxox-8 (10-fold dose) orally, on d 18 and 20 chicks were gavaged with 1 mL of a cocktail containing C. perfringens inoculations (4 x 10⁸ CFU). Culture of C. perfringens was obtained commercially (MicroBiotics, Cloud, MN, USA) and was propagated under anaerobic conditions for 16 hours at 37°C in screw cap tubes, cells were harvested by centrifugation (4500 g at 4°C) and diluted in physiological saline. Typical isocarlic and isonitrogenous starter (0-16 d) and finisher (17-30 d) diets based on corn-soybean meal diets were formulated in mashed form according to Table 1, which met or exceeded the recommendations in commercial practice in Saudi Arabia. Ambient temperature and relative humidity were concurrently and continuously recorded at 3-hour interval using two data loggers (HOBO Pro Series Data Logger, Model H08-032-08, Onset Co., Cape Cod, MA, USA) placed inside the chamber. The average temperature and relative humidity for the whole period were 24.95°C±0.26 (SD) and 26.63%±3.30 (SD), respectively. The study was conducted in April and May, 2012 under a protocol approved by King Saud University and complies with the current laws of animal protection in Saudi Arabia.

Measurements

Feed consumption and body weight gain (BWG) were recorded weekly by pen and feed conversion ratio (FCR) computed at 16 and 30 d. Mortality was checked daily and weights of dead birds were used to adjust FCR. At the conclusion of the trial at 30 d, five birds per treatment were selected, after euthanasia, feather, heads, necks, and shanks were removed, and the remaining carcasses were dissected to breast and leg quarter and were weighed. The percentage of yield of each part was calculated on the basis of dressed weight.

Histopathology and morphometric measurements

At 16 and 30 d, the entire gastrointestinal tract from five birds per treatment was removed aseptically, small intestine was weighed and the total length was measured, then was separated into duodenum, jejunum and ileum and for each part measurements of length and weight were taken. A 2-cm-long sample from each portion of the small intestine was collected for histology measurements. Samples were fixed in phosphate-buffered formalin for at least 48 h, after which they were embedded in paraffin. Sections of 5 mm were cut and stained with haematoxylin and eosin. Measurements of height and width were based on at least 5 well-oriented villi per section per broiler using an IX71 Inverted Olympus Microscope (Eyepiece: WH10X, Objective Lens: 4X) and a PC-based image analysis system (Olympus DPT2 Microscope Digital Camera; Olympus NV, Aartselaar, Belgium) with software Analysis (Cellens Digital Imaging Software for Research Application).

Enumeration and identification of bacterial cells

Ileal digesta contents were aseptically emulsified in a new sterile bag and kept in ice until time of analyses. Samples were weighed and diluted in 0.9% saline proportionally, and 0.1 mL of each sample was plated on duplicates by using selective agar media for enumeration of target bacterial groups. C. perfringens was counted on tryptose sulfate-cycloserine (TSC) agar for C. perfringens (Oxoid CM587 with the addition of SR88 and SR47). Colonies on TSC agar that were suspected to be C. perfringens were plated secondarily on blood agar (Garrido et al., 2004). Enterobacteriaceae were isolated on MacConkey agar (Oxoid CM7) after an incubation time of 24 h in an aerobic atmosphere at 37°C (Garrido et al., 2004). Isolates of Enterobacteriaceae and Salmonella were identified by API 20E. The API 20E strips (bioMérieux, Craponne, France) were inoculated, incubated at 37°C for 24 h and interpreted as recommended by the manufacturer. Reactions were recorded and identifications were determined by using a computer program [API Lab Plus software version 3.2.2 (bioMérieux)]. Results were expressed as log10 colony-forming units per ml of ileal digesta (log10 CFU g⁻¹).

Statistical analysis

All statistical analysis was performed using the Statistical Analysis System (SAS, 2002). A cage constituted the experimental unit. Four treatments were replicated 5 times in a random-

Table 1. Dietary composition of broiler chick starter and finisher diets.

| Treatment | 1 & 2 | 3 | 4 |
|-----------|-------|---|---|
| Starter   |       |   |   |
| Ingredients, % |       |   |   |
| Yellow corn | 56.00 | 55.99 | 55.95 |
| Soybean meal | 36.10 | 36.10 | 36.10 |
| Palm oil | 3.80 | 3.80 | 3.80 |
| DCP | 2.50 | 2.50 | 2.50 |
| Ground limestone | 0.72 | 0.72 | 0.72 |
| Choline chloride | 0.10 | 0.10 | 0.10 |
| DL-methionine | 0.23 | 0.23 | 0.23 |
| L-lysine | 0.15 | 0.15 | 0.15 |
| Salt | 0.50 | 0.30 | 0.30 |
| Vitamin premix | 0.05 | 0.05 | 0.05 |
| Trace mineral mix | 0.01 | 0.01 | 0.01 |
| Mannan | - | - | 0.05 |
| Total | 100 | 100 | 100 |
| Calculated analysis |       |   |
| ME, kcal/kg | 3000 | 3000 | 3000 |
| Crude protein, % | 22.0 | 22.0 | 22.0 |
| Non phytate P, % | 0.45 | 0.45 | 0.45 |
| Calcium, % | 1.0 | 1.0 | 1.0 |
| Lysine, % | 1.25 | 1.25 | 1.25 |
| Methionine, % | 0.55 | 0.55 | 0.55 |
| starter |
| Finisher |       |   |   |
| Ingredients, % |       |   |   |
| Yellow corn | 57.75 | 57.74 | 57.70 |
| Soybean meal | 34.0 | 34.0 | 34.0 |
| Palm oil | 4.80 | 4.80 | 4.80 |
| DCP | 2.0 | 2.0 | 2.0 |
| Ground limestone | 0.64 | 0.64 | 0.64 |
| Choline chloride | 0.05 | 0.05 | 0.05 |
| DL-methionine | 0.16 | 0.16 | 0.16 |
| L-lysine | - | - | - |
| Salt | 0.30 | 0.30 | 0.30 |
| Vitamin premix | 0.25 | 0.25 | 0.25 |
| Trace mineral mix | 0.05 | 0.05 | 0.05 |
| Mannan | - | - | - |
| Total | 100 | 100 | 100 |
| Calculated analysis |       |   |
| ME, kcal/kg | 3100 | 3100 | 3100 |
| Crude protein, % | 21.0 | 21.0 | 21.0 |
| Non phytate P, % | 0.40 | 0.40 | 0.40 |
| Calcium, % | 0.9 | 0.9 | 0.9 |
| Lysine, % | 1.1 | 1.1 | 1.1 |
| Methionine, % | 0.47 | 0.47 | 0.47 |

* Diet 3 had 0.01% Enramycin, diet 4 had 0.05% Safmannan on the expense of corn during starter and finisher. *Vitamin mix is supplied in the following per kg of diet: retinyl acetate, 3.41 mg; cholecalciferol, 0.87 mg; DL-α-tocopheryl acetate, 27.5 mg; menadione sodium bisulphate, 5 mg; riboflavin, 7.7 mg; niacin, 44 mg; pantothenic acid, 11 mg; cyanocobalamin, 0.02; choline, 496 mg; folic acid, 1.32 mg; pyridoxine HCl, 4.82 mg; thiamin mononitrate, 2.16 mg; D-biotin, 0.11 mg. °Trace mineral mix is supplied in the following per kg of diet: manganese, 67 mg; zinc, 54 mg; copper, 2 mg; iodine, 0.5 mg; iron, 75 mg; and selenium, 0.2 mg.
ized complete block design. Means for measurements showing significant differences in the analysis of variance were tested using the PDIF option. Means ± standard error of the mean (SEM) are presented in the tables and differences were considered statistically significant at P<0.05.

### Results

Performance observations at 16 and 30 d are listed in Table 2. During the starter period, BWG, FI and FCR were not influenced (P>0.05) by treatment. During the finisher period, no significant differences in FI was observed however, BWG was affected by dietary treatment (P<0.05); birds which had received the +CONT had the lowest BWG as compared to all other treatments but it was similar to those which had received +CONT. On the other hand, birds which had received ENRA or SAF had better FCR as compared to +CONT OR –CONT (P<0.01). During the cumulative period (0 to 30 d) birds which had received ENRA or SAF gained more weight as compared to those which had received the -CONT treatment (P<0.01). Also, cumulative FCR was affected by treatment (P<0.01); birds which had received ENRA or SAF had the best FCR among all groups. ENRA or SAF supplementation to the challenged birds resulted in 16 points improvement in FCR as compared to the challenged birds without medications (-CONT). No difference in cumulative FI due to treatment was observed. The mean percentage of carcass parts in different treatments is documented in Table 3. No difference in dressing percentage, breast muscle yield, leg quarter yield or abdominal fat was noticed between treatments (P>0.05).

The morphometric measurements of the intestinal epithelium samples at 16 d are given in Table 4. Ileal villus height was longer from birds which had received the +CONT or SAF as compared to the other treatments (P<0.05). Intestine length, weight and relative weight were not affected by any treatment (P>0.05). The morphometric measurements of the intestinal epithelium samples at 30 d are given in Table 5. Shorter small intestine was obtained from birds which had received +CONT or SAF as compared to all other treatments (P<0.05). On the other hand, birds which had received ENRA had the lowest duodenal length percent as compared to all other treatments (P<0.001). Ileal villus height and width were similar among all treatments (P>0.05).

Data related to ileal bacterial counts in broilers at 16 and 30 d are presented in Table 6. Similar bacterial count of *C. perfringens* and gram negative *Bactillus* were found in the starter period (before the challenge). At the end of the experiment, the ileal *C. perfringens* counts in the SAF birds were lowest among all groups (P<0.05). Populations of *C. perfringens* were reduced by 5 logs in the group which had received SAF as compared to the unmediated group (-CONT). No differences in gram negative *Bactillus* were found because of the treatment.

### Table 2. Body weight gain, feed intake and feed conversion ratio of broiler chickens given experimental diets at different ages.

| Treatment   | SEM | P   |
|-------------|-----|-----|
| **Performance 0-16 d** |     |     |
| BWG, g      | 474.7 | 482.7 | 517.2 | 499.6 | ±16.1 | ns  |
| FI          | 668.2 | 663.4 | 697.7 | 681.6 | ±16.1 | ns  |
| FCR, g:g    | 1.414 | 1.375 | 1.349 | 1.366 | ±0.03 | ns  |
| **Performance 17-30 d** |     |     |
| BWG, g      | 830.6² | 784.0³ | 856.7⁴ | 847.0⁴ | ±16.9 | *  |
| FL          | 1.521.4 | 1540.2 | 1461.6 | 1425.5 | ±51.2 | ns  |
| FCR, g:g    | 1.855a | 1.964a | 1.706c | 1.681b | ±0.05 | **  |
| **Cumulative 0-30 d** |     |     |
| BWG, g      | 1295.3bc | 1266.7c | 1373.9a | 1347.5ab | ±19.47 | **  |
| FL          | 2189.6 | 2203.6 | 2159.2 | 2107.0 | ±50.9 | ns  |
| FCR, g:g    | 1.682a | 1.734a | 1.571b | 1.565b | ±0.04 | **  |

| T1                  | T2                  | T3                  | T4                  |
|---------------------|---------------------|---------------------|---------------------|
| Performance 0-16 d  |     |     |     |
| BWG, g              | 474.7 | 482.7 | 517.2 | 499.6 |
| FL                  | 668.2 | 663.4 | 697.7 | 681.6 |
| FCR, g:g            | 1.414 | 1.375 | 1.349 | 1.366 |
| Performance 17-30 d |     |     |     |
| BWG, g              | 830.6² | 784.0³ | 856.7⁴ | 847.0⁴ |
| FL                  | 1.521.4 | 1540.2 | 1461.6 | 1425.5 |
| FCR, g:g            | 1.855a | 1.964a | 1.706c | 1.681b |
| Cumulative 0-30 d   |     |     |     |
| BWG, g              | 1295.3bc | 1266.7c | 1373.9a | 1347.5ab |
| FL                  | 2189.6 | 2203.6 | 2159.2 | 2107.0 |
| FCR, g:g            | 1.682a | 1.734a | 1.571b | 1.565b |

T1, unmedicated diet, unchallenged birds (+CONT); T2, unmedicated diet, birds were challenged with *C. perfringens* (-CONT); T3, 0.1 g/kg enramycin was added to the diet, birds were challenged with *C. perfringens* (ENRA); T4, 0.5 g/kg SAF-Mannan was added to the diet, birds were challenged with *C. perfringens* (SAF). BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

**Table 3. Effect of different treatments on parts yield as percentages of broiler dressed weight at d 30.**

| Treatment   | SEM | P   |
|-------------|-----|-----|
| Dressed yield, % | 60.9 | 60.7 | 62.1 | 59.8 | ±1.38 | ns  |
| Breast*, %    | 35.6 | 35.2 | 34.2 | 35.2 | ±0.77 | ns  |
| Leg quarter*, %| 40.4 | 40.3 | 40.2 | 40.9 | ±0.66 | ns  |
| Abdominal fat, %| 1.05 | 0.80 | 1.26 | 1.09 | ±0.17 | ns  |
| Liver, g/100 g| 0.31 | 0.24 | 0.28 | 0.32 | ±0.02 | ns  |

T1, unmedicated diet, unchallenged birds (+CONT); T2, unmedicated diet, birds were challenged with *C. perfringens* (-CONT); T3, 0.1 g/kg enramycin was added to the diet, birds were challenged with *C. perfringens* (ENRA); T4, 0.5 g/kg SAF-Mannan was added to the diet, birds were challenged with *C. perfringens* (SAF). *Breast and leg quarter were expressed as percentage of the carcass weight; ns, not significant.

**Table 4. Intestinal morphology and histology of broilers at d 16.**

| Treatment   | SEM | P   |
|-------------|-----|-----|
| Intestine length, cm | 129.7 | 132.0 | 127.0 | 128.3 | ±7.20 | ns  |
| Intestine weight, g/cm | 0.28 | 0.21 | 0.27 | 0.28 | ±0.05 | ns  |
| IRW, g/100g BW | 7.4 | 7.6 | 7.2 | 9.4 | ±0.51 | *  |
| Ileal villus height*, µm | 4500a | 3765b | 3804a | 4253b | ±185.8 | *  |
| Ileal villus width*, µm | 693 | 766 | 653 | 691 | ±50.8 | ns  |

T1, unmedicated diet, unchallenged birds (+CONT); T2, unmedicated diet, birds were challenged with *C. perfringens* (-CONT); T3, 0.1 g/kg enramycin was added to the diet, birds were challenged with *C. perfringens* (ENRA); T4, 0.5 g/kg SAF-Mannan was added to the diet, birds were challenged with *C. perfringens* (SAF). *Measurements of height and width were based on at least 5 well-oriented villi per ileum per broiler for a total of 5 birds per treatment. *Means in the row with different superscripts differ significantly. *P<0.05; **P<0.01; ns, not significant.
**Discussion**

The results revealed a significant improvement in FCR at 30 d for birds which had received the ENRA or SAF treatments. This could be explained by the significant improvement in BWG which was associated with birds which had received ENRA or SAF, while FI was similar among all groups. These results are in line with the findings of Podmaniczky et al. (2006); Rosen (2007), both groups reported a positive effect for yeast cell wall products on the performance of broilers. Birds which were subjected to the *C. perfringens* challenge without any medication (−CONT) had the highest FCR but not significantly different from the +CONT. According to Porter (1998) *C. perfringens* are counted among the most gut-specific pathogens which are assumed to be the main health problem associated with removing the antibiotics from feed. *C. perfringens* infection of broilers may cause impairment of production performance (Lovland and Kaldhusdal, 2001) and subclinical disease associated with necrotic enteritis which is characterized by damage to the intestinal mucosa that decreases digestion, absorption and reduces weight gains (Kaldhusdal et al., 2001).

On the other hand, the improvement in BWG and FCR could be also related to the lower microbial population in the gastrointestinal tract in broilers (Thongsong et al., 2008). Wilson et al. (2005) explained that the growth suppressing effect of intestinal bacteria was due to the production of toxic metabolites that irritate the gut mucosa, thereby inhibiting nutrient absorption. The ileal *C. perfringens* populations of birds were altered when SAF-Mannan was added to their diets. This came in accordance with the findings of Spring et al. (2000), Yang et al., (2007, 2008), where SAF-Mannan was found to have a positive effect in lowering *C. perfringens* populations. The significant reduction of ileal *C. perfringens* reported in this study could be explained by the ability of MOS to bind with pathogens in the small intestine by offering competitive binding sites for undesirable pathogens (Newman, 1994). MOS is not digested in the small intestine therefore; bacteria bound to MOS are likely exit the intestine without attaching to the epithelium and this cause a reduction or prevention of colonization of undesirable bacteria in the small intestine (Spring et al., 2000). The gross examination of the responses in birds challenged orally with *C. perfringens* showed sub-clinical inflammatory responses throughout various sections of gizzard, duodenum, jejunum, ileum and ceca associated with intestinal lesions and hemorrhages. This observation may partly explain the poor performance of the −CONT group.

Histological examination revealed a significant difference in villus height between the treatment groups at 16 d but not at 30 d. Birds which had received SAF improved villus height to a level which was similar to the unchallenged birds (+CONT). This indicated changes occurred in villi morphology between treatments during the starter period, and thus absorptive surface area in the small intestine and superior gut health. Several other reports observed greater villus height and superior ileal mucosa development in chickens supplemented with a yeast cell wall product prepared from *Saccharomyces cerevisiae*, particularly during the first week of a chicken’s life (Santin et al., 2001; Zhang et al., 2005). However, Yang et al. (2007) and Brümmer et al. (2010) reported no effect of MOS supplementation on gut morphology in chickens.

### Table 5. Intestinal morphology and histology of broilers at d 30.

| Treatment | T1 | T2 | T3 | T4 |
|-----------|----|----|----|----|
| SEM       | ±0.16 | ±0.32 | ±0.57 | ±0.16 |
| P         | ns | ns | * | ns |

| Intestine length, cm | 152.0 ‡ | 178.0 ‡ | 176.2 ‡ | 181.7 ‡ |
|----------------------|----------|----------|----------|----------|
| Duodenum length, %   | 15.7±a   | 16.6±a   | 14.8±a   | 15.3±a   |
| Jejunum length, %    | 52.1     | 41.8     | 43.2     | 40.5     |
| Ileum length, %      | 32.2     | 41.7     | 42.1     | 44.2     |
| Intestine weight, g/cm | 0.36 | 0.44 | 0.49 | 0.44 |
| IRW, g/100g BW       | 4.7      | 5.7      | 5.5      | 6.0      |

**Notes:** ‡ Measurements of height and width were based on at least 5 well-oriented villi per section per broiler for a total of 5 birds per treatment. a,b,c Means in the row with different superscripts differ significantly. *P<0.05; ns, not significant.

### Table 6. Ileal bacterial count mean, (log10 CFU/g) in broilers at d 16 and 30.

| Treatment | T1 | T2 | T3 | T4 |
|-----------|----|----|----|----|
| SEM       | ±0.16 | ±0.32 | ±0.57 | ±0.16 |
| P         | ns | ns | * | ns |

|          | T1 | T2 | T3 | T4 |
|-----------|----|----|----|----|
| Starter   | 4.2 | 3.9 | 4.3 | 4.2 |
| *C. perfringens* | 3.8 | 4.3 | 4.3 | 3.6 |
| Finisher  | 4.7 | 6.0 | 4.8 | 1.0 |
| *C. perfringens* | 4.8 | 4.7 | 5.0 | 4.7 |

**Notes:** ‡ Measurements of height and width were based on at least 5 well-oriented villi per section per broiler for a total of 5 birds per treatment. a,b,c Means in the row with different superscripts differ significantly. *P<0.05; ns, not significant.
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