Assessment of Refined Functional Carbohydrates as Substitutes of Antibiotic Growth Promoters in Broilers: Effects on Growth Performance, Immune Responses, Intestinal Microflora and Carcass Characteristics

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Abstract

The objective was to evaluate yeast based prebiotics, in solid and liquid forms separately, as replacement of antibiotic growth promoters in broiler chickens. Basal starter (ME: 3000 kcal/kg; CP: 22%) and finisher (ME: 3200 kcal/kg; CP: 20%) diets were formulated to serve as control (CONT). Basal diet was fortified with either Zinc Bacitracin 10% (ZNBC) or Enramycin 4% (ENRA) at 500 and 250 g/ton, respectively; while solid prebiotics were supplemented at 50 (SP50) or 100 g/ton (SP100). Liquid form of prebiotics was given at 0.5 (LP 0.5) or 1.0 ml per liter (LP1.0) in drinking water. Starter and finisher diets were fed to six replicates of 12 broiler chicks each, from day 1-21 and 22-35, respectively. Total feed consumption of broilers on diets containing prebiotics was higher (P<0.05) than those given control diet during starter (1183 vs 1178 g/bird). In finisher phase, birds given diets supplemented with prebiotics had higher feed intake than those given antibiotics (2241 vs 1996 g/bird). Weight gain (1883 vs 1532 and 1604 g/bird), feed conversion ratio (FCR; 1.83 vs 2.08 and 2.11) and European Production Index (EPI; 291 vs 181 and 205) were better in prebiotics fed birds than on antibiotics or control during overall period. Inclusion of antibiotics and prebiotics in broiler diet resulted in lower Escherichia coli (E. coli) and Salmonella Spp. count than in control group (0.24 and 1.16 vs 1.53 10⁴ CFU/g and 0.15 and 0.42 vs 1.03 10⁵ CFU/g, respectively). Dressing percentage was better (59.6 vs 57.9%; P<0.05) in birds given prebiotics than those on antibiotics. In conclusion, refined functional carbohydrate may be used as an alternative to antibiotics in broilers. Furthermore, liquid prebiotics seemed more effective than solid prebiotics in enhancing the growth performance during starter phase.

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Introduction

Purpose of using antibiotic growth promoters (AGPs) in broiler nutrition is to control the intestinal pathogens, resulted in better performance of birds (Dibner and Richards, 2005). Although, because of increasing consumers’ concerns for drug residues in poultry products, like eggs and meat (Mathur and Singh, 2005), use of AGPs in animals’ diet was banned in 2006 by European Union (EC, 2003). Thus, many substances were tested as replacement of antibiotics in poultry feeding that could maintain chicken health with no residual effect on the health of consumers. Examples of such substances include prebiotics, probiotics, phenolic compounds and acidifiers (Baurhoo et al., 2009; Buddington, 2009).

Prebiotic are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in colon, and thus improve host health (Butel and Waligora-Dupriet, 2016). Prebiotics directly target colon, have a selective fermentation and help maintaining a
balanced microflora, preferably by being utilized by promoting species and increased excretion of pathogens through faeces. In addition to local effects, other systemic effects may also occur after absorption of fermentation products into blood stream (Sinovec and Markovic, 2005). Yeast and its products (yeast extracts, yeast culture, and whole yeast cells) have been reported to improve nutrient utilization and growth in broilers (Gomez and Angeles, 2011). Supplementation of yeast (Saccharomyces cerevisiae) resulted in improved growth and carcass weight, and lower abdominal fat in broiler chicks (Onifade et al., 1999).

Celmanax™ is a yeast culture product with refined functional carbohydrates, harvested by enzymatic hydrolysis of yeast (Saccharomyces cerevisiae). It contains complex sugars like mannose, β-glucans, mannan oligosaccharides (MOS) and galactosamine that work as prebiotics. The MOS and β-glucans have positive effects in modulating gut health of animals and immune responses and results in better production performance by birds (Gao et al., 2008). The MOS are reported as appropriate substitutes of AGPs in broiler chickens (Mohamed et al., 2008). Gomez et al. (2012) also reported that dietary inclusion of enzymatically hydrolyzed yeast resulted in better feed efficiency in broiler chickens.

The objective of this research was to compare the efficacy of commercial yeast based prebiotics, in solid and liquid forms, as alternatives of antibiotic growth promoters and evaluate their influences on growth performance, intestinal microflora, immune responses and carcass parameters in broilers.

MATERIALS AND METHODS

Bird husbandry: Five hundreds and four day-old broiler chicks (Hubbard classic strain) were procured from a commercial hatchery. The chicks were reared under standard husbandry conditions. The chicks were then, divided into 42 replicates of 12 birds each at random and placed in separate pens. Saw-dust was used as bedding material. House temperature was set at 95°F during 1st week and then reduced by 5°F each week till it reached 75°F. Birds were vaccinated against Infectious bronchitis and Newcastle disease (on 3rd and 25th days, respectively), and infectious bursal disease (on 13th and 20th days). Experiment was conducted in compliance with ethical standards of animal care of Institutional Biosafety Committee, University of Agriculture Faisalabad, Pakistan.

Experimental diets: Basal starter (ME: 3000 kcal/kg; CP: 22%) and finisher (ME: 3200 kcal/kg; CP: 20%) diets were formulated to serve as control (Table 1). Basal diet was fortified with either Zinc Bacitracin 10% (ZNBC) or Enramycin 4% (ENRA) at 500 and 250 g/ton, respectively; while solid prebiotics were supplemented at 50 (SP50) or 100 g/ton (SP100). Liquid form of prebiotics was given at 0.5 ml (LP 0.5) or 1.0 ml per liter (LP1.0) in drinking water. Starter and finisher diets were fed to six replicates of 12 birds each, from day 1-21 and 22-35, respectively.

Sampling and measurements: Feed intake and body weight were recorded on weekly basis. Mortality was recorded and weights of the dead broiler birds were accounted to measure the corrected feed intakes, feed conversion ratio (FCR) and European Production Index (EPI).

Immune responses: Blood samples were collected from wing vein of two birds from each replicate. Blood sample were transferred to gel-activated tubes and serum samples were collected. The supernatant serum was collected, placed in plastic eppendorf tubes. For immune responses, antibody titer against Newcastle disease virus (NDV) was measured by hemagglutination-inhibition test by the method of OIE (2012) and against infectious bursal disease virus (IBDV) by using ELISA technique as described by Alkhalaf (2009).

Enumeration of intestinal microflora: At the end of experiment, three birds per replicate were placed in a separate pen with cleaned plastic sheet and fresh fecal samples of each replicate were collected under aseptic conditions. Then, 1/10 serial dilutions of excreta were prepared in buffered peptone water (1g/L peptone, 8g/L NaCl, and 500 mg/L L-cysteine hydrochloride).

Nutrient and Rogosa agars were used for total bacterial and Lactobacilli counts, respectively. Rapid E. coli 2 agar and E. coli supplement was used to quantify E.coli. For Salmonella Spp. count, dilutions were multiplied in tetrathionate broth for 24 h and then cultured on modified brilliant green agar with 20 g/L novobicin.

The culture plates for total bacterial count, E. coli and Salmonella Spp. were incubated at 37°C in an aerobic environment whereas for Lactobacillus at 30°C in a microaerobic environment for 24 hours. For each of these bacteria, cfu in log10 per gram of excreta was counted on the basis of characteristics and morphology of colony.

Carcass parameters: For carcass trait assessment, two birds per replicate were randomly selected, weighed individually and slaughtered. Dressing (% of live weight), breast and thigh meat percentages, and giblet weights (% of carcass weight), were calculated.

Statistical analysis: Data were analyzed with General Linear Models of SAS 9.1 (SAS, 2010) using Completely Randomized Design and orthogonal contrasts were used for means comparison.

RESULTS

Performance of broiler chickens during starter phase (0-3 week): Total feed consumption increased (P<0.05) in broilers given antibiotics and prebiotics supplemented diets than those given control diet during starter phase. Better weight gain, FCR and EPI was recorded in prebiotics supplemented groups than those fed antibiotics or control supplemented diets. Higher weight gain (P<0.05), better FCR and EPI (P<0.05) were observed by birds given liquid prebiotics than those fed solid prebiotics. Mortality was lower (P<0.05) in birds on control and antibiotic supplemented diets than on prebiotics; birds given solid or liquid prebiotics had similar (P>0.05) mortality rate in broiler birds (Table 2).
**Table 1: Ingredients and nutrients composition of experimental diets**

| Ingredient (%) | Starter | Finisher |
|----------------|---------|----------|
| Maize          | 57      | 62       |
| Soybean meal   | 33      | 26.35    |
| Corn gluten 60 | 2.0     | 3.0      |
| Chips (CaCO₃)  | 1.3     | 1.0      |
| Di calcium phosphate | 2.1 | 2.2 |
| Salt (NaCl)    | 0.45    | 0.45     |
| Lysine HCl     | 0.4     | 0.2      |
| Di-Methionine  | 0.3     | 0.25     |
| Canola oil     | 3.0     | 4.0      |
| L-Threonine    | -       | 0.1      |
| Sodium bi carbonate | 0.1 | 0.1 |
| Mineral Premix  | 0.2 | 0.2 |
| Vitamin Premix** | 0.25 | 0.35 |

**Calculated nutrients composition (%)**

- **Dry matter**: 88.8, 88.8
- **Crude protein**: 22.2, 20.0
- **Metabolizable energy, kcal/kg**: 3011, 3203
- **Ether extract**: 5.4, 6.6
- **Crude fiber**: 3.6, 3.4
- **Ash**: 5.6, 5.1
- **Calcium**: 1.0, 0.9
- **Available phosphorus**: 0.5, 0.5
- **Sodium**: 0.2, 0.2
- **Chlorine**: 0.4, 0.4
- **Lysine**: 1.4, 1.1
- **Methionine**: 0.6, 0.6
- **Methionine + Cystine**: 1.0, 0.9
- **Threonine**: 0.8, 0.8
- **Tryptophan**: 0.3, 0.2
- **Isoleucine**: 0.9, 0.8
- **Valine**: 1.0, 0.9
- **Arginine**: 1.4, 1.2

**Analyzed nutrients composition (%)**

- **Dry matter**: 89.9, 91.2
- **Crude protein**: 21.5, 20.2
- **Ether extract**: 4.7, 5.8
- **Crude fiber**: 5.5, 5.6
- **Ash**: 6.0, 5.7

*Mineral premix provides 30 mg Fe, 50 mg Zn, 5 mg Cu, 60 mg Mn, 0.1 mg Co, 0.3 mg I, and 1 mg Se per kg of diet. **Vitamins premix provides 10000 IU Vitamin A, 5 mg Riboflavin, 12 mg Ca Panthenolate, 2.2 mg thiamin, 1.55 mg Folic acid, 44 mg nicotinic acid, 2.2 mg Vitamin B6, 12.1 μg Vitamin B12, 250 mg Choline chloride, 0.11 mg d-biotin, 1100 IU Vitamin D3, 11.0 IU Vitamin E. 1.1 mg Vitamin K per kg of diet.

**Performance of broiler chickens during finisher phase (4-5 week):** Feed consumption increased (P<0.05) in prebiotics supplemented groups than those given antibiotics supplemented diets during finisher phase. Weight gain was improved in birds fed prebiotics supplemented diets than on control and on antibiotics supplemented diets. The FCR was not affected (P>0.05) by antibiotics or prebiotics dietary supplementation in broiler diets, however, it was poorest (P<0.05) in control group. Mortality was lower (P<0.05) in birds on control and prebiotics supplemented diets than on antibiotics; however, birds fed liquid prebiotics had lower (P<0.05) mortality than those on solid probiotics. The EPI was better in prebiotics supplemented birds than all other treatments (Table 2).

**Performance of broiler chickens during overall period (0-5 week):** Total feed consumption during overall period was higher (P<0.05) in prebiotics supplemented groups than antibiotics supplemented groups (Table 2). Prebiotics supplementation resulted in improved (P<0.05) weight gain, FCR and EPI than all other treatments. Higher mortality rate was noted in birds offered control diet than those offered antibiotics or prebiotics supplemented diets (Table 2).

Immune responses: Antibodies titers against NDV and IBDV were not statistically different (P>0.05) in broilers fed diets with or without prebiotics or antibiotics at 19th and 32nd day of age (Table 3).

**Intestinal micro-flora:** Higher total bacterial count (P<0.05) was observed in birds fed diets containing prebiotics than those fed antibiotics. Likewise, higher *E. coli* and *Salmonella* Spp. counts (P<0.05) were recorded in birds fed diet containing prebiotics than those on antibiotics. *Lactobacillus* count was improved (P<0.05) in birds on prebiotics than those on antibiotics (Table 3).

**Carcass characteristics:** Dressing percentage was improved (P<0.05) in prebiotics groups than antibiotics groups, while higher (P<0.05) thigh meat yield was recorded in birds given antibiotics than those fed prebiotics supplemented diets (Table 3). Breast meat yield and abdominal fat were not influenced (P>0.05) by dietary treatments.

**DISCUSSION**

Feed intake, weight gain, FCR and EPI was improved in birds fed prebiotics supplemented diets than those given and control antibiotics supplemented diets. Results of this study are in accordance with the published literature as Santin *et al.* (2001) reported that supplementation of yeast cell walls resulted higher feed intake in broilers than on control diets. Similarly, Abdel-Hafeez *et al.* (2017) reported that feed consumption in the prebiotic group was higher than in control group in broilers. However, Baurhoo *et al.* (2009), Eseceli *et al.* (2010) and Wang *et al.* (2018) reported that feed intake was not influenced by dietary supplementations of AGPs and prebiotics in broilers. The results of the present findings regarding weight gain and FCR are in agreement with the findings of Shahir *et al.* (2014) who reported improved weight gain and FCR in broiler birds fed diet containing yeast based prebiotics. Similarly, Abdel-Hafeez *et al.* (2017) found that chicks fed diets supplemented with prebiotic (with and without feed restriction) exhibited higher body weight and feed efficiency than chicks fed the control diets. Improvement in performance of broiler birds in terms of weight gain, FCR and EPI by dietary supplementation of prebiotics, might be due to improvement of health of the intestinal flora and to better absorption of dietary nutrients in gut (Santin *et al.*, 2001). Mohamed *et al.* (2008) concluded that prebiotics supplementation had same effects on weight gain (263 vs. 264 g/bird) and FCR (1.40 vs. 1.40) in broiler chicken compared with Enramycin. However, Kamran *et al.* (2013) reported that broiler birds fed diets containing antibiotics (Zinc bacitracin, Enramycin and Furazolidone) had better weight gain (851, 863 and 862 vs. 821 g/bird), FCR (1.47, 1.48 and 1.49 vs. 1.53) than those on diet containing prebiotics during starter phase. In contrast, Sarangi *et al.* (2016) also concluded that broiler birds offered prebiotics supplemented diets had lower body weight compared to control groups. These variations in results may be due to differences in source and nature of yeast products.
### Table 2: Effect of replacing antibiotics with refined functional carbohydrate on growth performance of broilers

| Parameters         | Experimental diets | SEM | P-Value (Orthogonal contrast) |
|--------------------|--------------------|-----|-------------------------------|
|                    | CONT | ZNBC | ENRA | SP50 | SP100 | LP0.5 | LP1.0 |      |      |
| **Start of phase** |      |      |      |      |      |       |       |      |      |
| **(0-3 weeks)**   |      |      |      |      |      |       |       |      |      |
| BWG, g            | 42.5 | 43.1 | 42.3 | 42.2 | 42.0 | 43.4  | 41.2  | 68   | NS  |
| FI, g              | 1118 | 1195 | 1161 | 1210 | 1159 | 1172  | 1190  | 24   | *   |
| EPI, %             | 2.8  | 2.8  | 2.4  | 2.8  | 1.4  | 6.9   | 1.4   | 1.6  | NS  |
| **(4-5 weeks)**   |      |      |      |      |      |       |       |      |      |
| BWG, g            | 2251 | 2111 | 1880 | 2305 | 2164 | 2251  | 2243  | 56   | *** |
| FI, g              | 947  | 1037 | 1037 | 1203 | 1086 | 1117  | 1091  | 46   | NS  |
| EPI, %             | 8.3  | 8.3  | 23.6 | 1.4  | 5.6  | 1.4   | 4.2   | 2.4  | NS  |
| **Overall period** |      |      |      |      |      |       |       |      |      |
| BWG, g            | 1604 | 1602 | 1461 | 1987 | 1771 | 1894  | 1880  | 54   | NS  |
| FI, g              | 2.1  | 2.0  | 2.0  | 1.7  | 1.8  | 1.8   | 1.8   | 0.05 | NS  |
| EPI, %             | 2.0  | 2.0  | 2.0  | 2.0  | 2.0  | 2.0   | 2.0   | 0.08 | NS  |

### Table 3: Effect of replacing antibiotics with refined functional carbohydrate on immune responses, intestinal micro-flora and carcass characteristics of broilers

| Parameters         | Experimental diets | SEM | P-Value (Orthogonal contrast) |
|--------------------|--------------------|-----|-------------------------------|
|                    | CONT | ZNBC | ENRA | SP50 | SP100 | LP0.5 | LP1.0 |      |      |
| **Immune responses**|      |      |      |      |      |       |       |      |      |
| ND Titer (GMT) at 19th day | 2.8  | 3.5  | 4.1  | 3.4  | 2.5  | 4.0   | 2.9   | -    | NS  |
| ND Titer (GMT) at 32nd day | 2.6  | 3.3  | 2.8  | 3.8  | 2.7  | 4.1   | 2.5   | -    | NS  |
| IBD Titer (OD) at 19th day | 254 | 85  | 108 | 102 | 75  | 103   | 97    | 70   | NS  |
| IBD Titer (OD) at 32nd day | 793 | 308 | 866 | 327 | 394 | 411   | 643   | 293  | NS  |
| **Intestinal micro-flora**|      |      |      |      |      |       |       |      |      |
| TBC, 10^5 CFU/g | 2.33 | 1.33 | 1.67 | 2.47 | 2.23 | 2.03  | 1.97  | 0.26 | NS  |
| E. coli, 10^5 CFU/g | 1.53 | 0.3  | 0.17 | 1.17  | 1.03 | 1.13  | 1.30  | 0.24 | NS  |
| Salmonella Sp., 10^5 CFU/g | 1.03 | 0.2  | 0.1 | 0.13  | 0.17 | 0.08  | 0.07  | 0.13 | *** |
| Lactobacillus, CFU/g | 2.0 | 1.33 | 2.0 | 4.0  | 3.3  | 3.0   | 3.6   | 0.69 | NS  |
| **Carcass characteristics**|      |      |      |      |      |       |       |      |      |
| Dressing percentage | 59.6 | 58.2 | 57.6 | 60.3 | 59.0 | 59.7  | 59.5  | 0.59 | NS  |
| Chest meat yield, % | 58.8 | 57.0 | 57.0 | 57.7 | 58.2 | 57.8  | 57.2  | 0.65 | NS  |
| Thigh meat yield, % | 42.1 | 42.6 | 43.2 | 42.3 | 41.8 | 42.1  | 41.1  | 0.54 | NS  |
| Fat pad, % | 36  | 3.4  | 3.4 | 3.1  | 3.6  | 2.9   | 3.5   | 0.22 | NS  |

CONT = Basal Diet; ZNBC = Basal diet supplemented with Zinc Bacitracin @ 500g/ton; ENRA = Basal diet supplemented with Enramycin @ 250g/ton; SP50 = Basal diet supplemented with Celmanax SCP @ 50g/ton; SP100 = Basal diet supplemented with Celmanax SCP @ 100g/ton; LP0.5 = Basal Diet + Celmanax Liquid @ 0.5ml/liter of drinking water; LP1.0 = Basal Diet + Celmanax Liquid @ 1.0ml/liter of drinking water; BWG = Body weight gain; FI = Feed intake; EPI = European production index; NS: non-significant (P>0.05); *: P<0.05; **: P<0.01; ***: P<0.001; ab Values not followed by a common superscript differ significantly (P<0.05).
used (active dried yeast, yeast cell wall, yeast auto lysate or beta-glucans; Yalcin et al., 2013). Lower mortality rate was noted in broilers fed control and antibiotic supplemented diets than those offered prebiotics during starter phase. However, it was lower in broilers offered control and prebiotics supplemented diets than those fed diets supplemented antibiotics during finisher phase. Higher mortality rate was noted in broilers offered diet supplemented with Enramycin, which may be because of nephrotoxicity caused by dietary supplementation of antibiotics during early life period of birds. Some researchers (Gao et al., 2008 and Morales-López et al., 2009) reported no effect of yeast based products on mortality rate in broiler birds.

Antibodies titers against NDV and IBDV were not statistically different in broilers fed diets with or without prebiotics or antibiotics at 19th and 32nd day of age. Results of this study are in accordance with the published literature as Salianeh et al. (2011) and Sadeghi et al. (2013) reported no effect of prebiotics supplementation on antibodies titers against NDV and IBDV in broilers. Similarly, Houshmand et al. (2012) and Shahir et al. (2014) also reported that prebiotics supplementation had no significant effect on antibody titer against NDV in broilers. In contrast, Gao et al. (2008) reported that linear increase (P<0.05) in antibodies titer against NDV with increasing level of yeast culture in diet which shows that humoral or systemic immunity can be affected by dietary yeast culture supplementation in broiler birds. These differences might be because of differences in forms and levels of prebiotics used.

Total bacterial count, E. coli and Salmonella Spp. counts were also higher (P<0.05) in broilers fed prebiotics than those offered antibiotics. However, prebiotics supplemented groups maintained the populations of potential pathogens (E. coli and Salmonella Spp.) at relatively low levels than the control group. Moreover, they also exerted a stimulatory effect on health-promoting bacteria (Lactobacillus). These results are in agreement with Yalcin et al. (2013) who concluded that total aerobic bacterial count and E. coli count was decreased by prebiotics supplementation. The results of the present findings regarding Lactobacillus count are in agreement with the findings of Biswas et al. (2018), who concluded that Lactobacillus count was higher (P<0.05) in yeast based prebiotic supplemented group than antibiotics supplemented and control group. This seem consistent with Baurhoo et al. (2009) who reported that the antibiotic was less effective in maintaining of the intestinal tissue healthiness and morphology than prebiotic due to its bad effect on the beneficial intestinal bacteria. Kim et al. (2011) also reported that feeding prebiotics increased the Lactobacillli populations and decreased the E. coli populations than control and antibiotics fed broiler birds. However, Nabizadeh (2012) reported that prebiotic inclusion had no effect on Lactobacilli and E. coli counts in broilers. Hahn-Didde and Purdum (2016) also observed no differences in viable counts of total aerobes, E coli, or Salmonella Spp. by prebiotics supplementation. These variations might be due to the differences in environment and facilities in which birds are reared.

Higher dressing percentage was noted in birds fed prebiotics supplemented diet than antibiotics diet. These results are in agreement with Gomez and Angeles (2011) who reported higher carcass yield (46.4% vs. 42.9%) in broilers fed diet containing enzymatically hydrolyzed yeast than those fed control diet. Onifade et al. (1999) reported better carcass yield (72.3% vs. 68.9%) in broiler fed diet containing dried yeast than control group. In contrast, Yalcin et al. (2014) and Abdel-Hafeez et al. (2017) reported no effect of prebiotics supplementation on carcass yield in broilers. Other carcass parameters like breast meat yield, and abdominal fat weights were not affected (P>0.05) by dietary treatments. These results are in line with Pelcica (2004), Gomez and Angeles (2011) who reported that breast meat yield and abdominal fat percentage were not influenced by inclusion of antibiotics or prebiotics in the diet of broiler birds. Similarly, Wang et al. (2014) and Shulukh el al. (2017) also concluded that prebiotics supplementation had no effect on breast muscle ratio and abdominal fat ratio in broilers.

Conclusions: Refined functional carbohydrates, in solid and liquid forms separately, were more effective in enhancing growth performance, dressing percentage and decreasing E. coli and Salmonella Spp. in broilers than the antibiotics growth promoters; therefore, can be used as an alternative to antibiotics in broiler nutrition. Moreover, liquid prebiotics were more effective than solid prebiotics in enhancing the growth performance during the starter phase.

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Authors contribution: SA did experimental work and manuscript writing; SAB performed data analysis; ZK did experimental work and manuscript preparation; SUR performed experimental trials on intestinal flora.

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