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# The effects of dietary supplementation with different levels of Microzist as newly developed probiotics on growth performance, carcass characteristics, and immunological organs of broiler chicks

Mohammad Parsa[a], Mehran Nosrati[a], Faramin Javandel[a], Alireza Seidavi[a], Ameer Khusro[b] and Abdelfattah Z. M. Salem [c]

[a]Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran; [b]Research Department of Plant Biology and Biotechnology, Loyola College, Chennai, India; [c]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, México

**ABSTRACT**

The current research was conducted to evaluate the influence of newly developed probiotic (Microzist) and commercial probiotic (Primalac) on growth performance, carcass characteristics, and immunity of broiler chickens. A total of 225 one-day-old broiler chicks Ross-308 was randomly assigned into 5 groups – control group, group with 0.2, 0.25, and 0.3 g/kg of Microzist, and group constituting Primalac (starter: 0.9 g/kg, grower: 0.454 g/kg, and finisher: 0.225 g/kg). At 42$^{nd}$ day of age, there were no statistically significant differences among treatments on daily weight gain and feed conversion ratio ($P > .05$), but treatment's effect on the feed intake was found to be significant ($P < .05$). Diet supplementation with various concentrations of Microzist showed growth performance of broiler chickens more or less similar to the Primalac additives. There were statistically significant differences ($P < .05$) among treatments on the carcass characteristics. Microzist (0.2 g/kg) reduced the abdominal fat content in broiler chickens in a comparison with control as well as Primalac ($P < .05$). Furthermore, Microzist showed no influence on the immunological organ weights. The present investigation indicates that Microzist may be a potential alternative to antibiotic growth promoters as well as widely used Primalac in broiler industries.

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Broiler chickens; carcass; immunity; Microzist; Primalac; performance

## Introduction

The use of specific feed additives is a remarkable parameter for the high-level productivity and efficient feed conversion in the modern broiler industry. For the past few years, different strategies have been adopted in order to significantly improve poultry productivity and profitability by maintaining health, reduced risk of possible side effects, and potentiality to improve the immunity. In this regard, antibiotics have been successfully used as growth promoters at sub-therapeutic doses in poultry feed (Goodarzi et al. 2014; Landy and Kavyani 2014). However, in the current scenario, limited applications of antibiotics in broiler industries came into account due to the emergence of antibiotic resistant bacteria and consumer's concern regarding food safety, which poses a potential threat to humans (Toghyani et al. 2010). The development of antibiotic resistant bacteria compelled the worldwide researchers to use suitable non-therapeutic and nutriceutical alternatives as feed additives in the broiler industry for preventing the proliferation of pathogens and modulating beneficial gut microorganisms in order to improve the health and performance.

Consequently, probiotics have received increased attention as possible replacement of antibiotic growth promoter in order to induce the growth, and further maximize the genetic prospective of modern broiler (Dhama et al. 2011). Probiotics have been defined as live microorganisms which when administrated in adequate amounts, favour intestinal microflora balance by stimulating their growth, and confer health benefit to a host (Mercenier et al. 2003). The probiotics inhibit the growth of gut pathogens by competitive exclusion antagonism, acid fermentation, bacteriocins production, stimulation of the immune system, competition for available nutrients, and adhesion receptors to intestinal epithelium (Lee et al. 2010).

Several reports had shown that probiotics in broiler's diets improve the growth performance as a replacement of antibiotic growth promoters (Shim et al. 2010; Wang and Gu 2010; Zakeri and Kashefi 2011). Primalac, a kind of commercial probiotic that contains at least $1 \times 10^8$ CFU $g^{-1}$ *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faesium* (Chichlowski et al. 2007a, 2007b) showed improvements in growth performance, feed efficiency, and immune response in broiler chickens (Samli et al. 2007; Rahimi et al. 2009; Landy and Kavyani 2014). The potentiality of probiotics may be improved by the selection of potent strains, gene manipulation, the combination of two or more strains, and the combination of probiotics as well as synergistically acting constituents that beneficially affects the host.

The quest of new, effective, and non-toxic feed additives on poultry growth against unexpected hazards is still continuing.

**CONTACT** Prof. Dr. A. Z. M. Salem asalem70@yahoo.com Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca 50000, México

From this point of view, in the present context, a significant attempt was undertaken to evaluate the performance of commercial broilers by administrating newly developed probiotics, Microzist. To the best of our knowledge, so far there is no research activity focussing on the application of Microzist in the broiler industry. Hence, the present study was conducted to compare the effect of different levels of dietary Microzist and a commercial probiotics (Primalac) on performance, immunity, and carcass characteristics of broilers during 6-weeks period and to ascertain the significance of Microzist on these responses.

## Materials and methods

### Chicks

The randomized experiment was carried out according to the methodology of Nosrati et al. (2017) with slight modifications. The experimental design consists of five treatments in three replicates. A total of 225 one-day-old Ross 308 male chicks (Aviagen, Newbridge, Scotland 35805, UK) were allotted to 15 groups, containing 15 birds in such a way that mean group body weights were similar for all groups.

### Preparation of the rearing house and maintenance of abiotic factors

Prior to the experiment, the facility, drinkers, and feeders were carefully cleaned and disinfected according to the methodology of Nosrati et al. (2017). In addition to this, abiotic factors such as temperature, humidity, and light were also maintained according to the instructions mentioned.

### Health programmes

Sanitation principles and health measures for raising chickens were applied. Drinkers were washed and cleaned daily. The birds were vaccinated against infectious bronchitis disease (d1 and d7), Newcastle disease (d1, d7, and d18), influenza disease (d1), and infectious bursal disease (d14 and d23).

### Experimental treatments

The experiments were carried out with five treatments as follows:

Treatment 1: Control diet without probiotics (from 1st to 42nd days of age).

Treatment 2: Diet included Microzist probiotics (0.2 g/kg from 1st to 42nd days of age).

Treatment 3: Diet included Microzist probiotics (0.25 g/kg from 1st to 42nd days of age).

Treatment 4: Diet included Microzist probiotics (0.3 g/kg from 1st to 42nd days of age).

Treatment 5: Diet included Primalac probiotics (starter: 0.9 g/kg, grower: 0.454 g/kg, finisher: 0.225 g/kg).

### Diet composition

All chickens were fed according to standard recommendation. The feed ingredients and calculated nutrient composition in the starter (1st–21st days of age) and finisher (22nd–42nd days of age) periods are shown in Tables 1 and 2.

### Performance, carcass characteristics, and immune responses

Feed intake and body weight were recorded for each phase and feed conversion ratio was calculated. At d42, after 4 h of fasting, one bird from each replicate was selected. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight and carcass yield. Distribution of meat and gastrointestinal tract characteristics were measured. Birds were plucked by dry plucking method. Feet were separated from the carcass in the tibiotarsal joint. Neck, wingtips, gut, and liver were removed and the empty or edible carcass was weighed, and intestinal segments dimensions were recorded. Various parts of the carcasses and immunity-related organs were also dissected and weighed separately.

### Statistical analyses

Data were analysed using a completely randomized experimental design involving five treatments. Data were subjected to statistical analysis using the General Linear Model procedures of the Statistical Analysis System v8 (SPSS 1997). Differences among main effect means ($P \leq .05$) were assessed via Duncan's multiple range tests. Statements of significance were verified based on $P \leq .05$.

**Table 1.** Feed ingredients of diets used during the starter (1st–14th days of age), grower (15th–28th days of age), and finisher (29th–42nd days of age) periods.

| Ingredient | Starter | Grower | Finisher |
|---|---|---|---|
| Corn | 543.2 | 600 | 640 |
| Soybean meal | 394.3 | 318.7 | 270.0 |
| Oyster shell | 9 | 7.9 | 10.0 |
| Corn oil | 21.6 | 45 | 50.0 |
| Di-calcium phosphate | 20.5 | 16.8 | 18.5 |
| Salt | 3.7 | 3.7 | 3.5 |
| DL-methionine | 2 | 2.2 | 1.8 |
| L-lysine | 0.7 | 0.5 | 1.2 |
| Vitamin mixture[a] | 2.5 | 2.5 | 2.5 |
| Mineral mixture[b] | 2.5 | 2.5 | 2.5 |
| Total | 1000 | 1000 | 1000 |

[a]Vitamin A (Retinol): 5000 IU/g; Vitamin D3 (Cholecalciferol): 500 IU/g; Vitamin E (Tocopherols): 3 mg/g; Vitamin K (Phylloquinone): 1.5 mg/g; Vitamin B2 (Riboflavin): 1 mg/g.
[b]Calcium pantothenate: 4 mg/g; Niacin: 15 mg/g; Vitamin B6: 13 mg/g; Cu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g.

## Results

### Effect of Microzist and Primalac on performance

Feed intake mean of Ross 308 broilers fed diets supplemented with different concentrations of Microzist and standard level of

**Table 2.** Nutrient analysis of diets used during the starter (1st–14th days of age), grower (15th–28th days of age), and finisher periods (29th–42nd days of age).

| Ingredient | Starter | Grower | Finisher |
|---|---|---|---|
| Energy (kcal/kg) | 2900 | 3200 | 3220 |
| Crude protein (g/kg) | 221.6 | 213.0 | 195.0 |
| Calcium (g/kg) | 10.0 | 8.5 | 10.3 |
| Available phosphorus (g/kg) | 5.0 | 4.2 | 5.8 |
| DCAB (mEq/kg) | 236 | 202 | 172 |
| Lysine (g/kg) | 11.5 | 9.6 | 11.2 |
| Methionine (g/kg) | 5.0 | 4.8 | 4.9 |
| Methionine + Cysteine (g/kg) | 8.3 | 7.8 | 7.3 |
| Threonine (g/kg) | 7.9 | 7.1 | 6.5 |

Primalac is summarized in Table 3. The feed intake for Microzist (0.2 and 0.3 g/kg) was higher than the control group during 1st–14th days of age. Microzist at the concentration of 0.25 g/kg showed significantly ($P < .05$) more or less similar feed intake values (486.0 g) compared to control (489.0 g) during starter period. The feed intake values for Microzist at all concentrations were not much impressive in a comparison with control during 15th–28th days of age. In contrary to this, Microzist at 0.25 g/kg tested here during 29th–42nd days of age showed higher feed intake values with respect to the control ($P < .05$). On the other hand, Primalac at standard level showed higher feed intake values than control and Microzist of all concentrations tested during starter, grower, and finisher periods. However, the feed intake for Primalac was found to be significantly ($P < .05$) higher (4246.0 g) than those of control and all concentrations of Microzist during a complete duration ($P < .05$).

Table 3 represents the weight gain mean of Ross 308 broilers fed diets supplemented with Microzist and Primalac from 1st to 42nd days of age. Diet supplementation with Microzist at the concentration of 0.2 and 0.3 g/kg did not increase chicks body weight during 1st–14th days and 15th–28th days of age relative to the control chicks. Results also showed that Microzist at the concentration of 0.2 and 0.25 g/kg did not induce the body weight during finisher period in a comparison with the control. The total weight gain mean during the total period of 1st–42nd days of age were found to be unaffected relative to the control broilers after the supplementation of Microzist. In like manner, Primalac supplemented group showed no influence on the body weight gain (1st–14th days = 24.92; 15th–28th = 42.19; 29th–42nd = 68.95; 1st–42nd days = 52.38) compared to the control as well as broilers fed with different concentrations of Microzist during the starter, grower, finisher, and total periods.

During the starter period, the incorporation of Microzist at all concentrations showed no significant elevation in the feed conversion ratio (1.40), compared to the control (1.34), and Primalac (1.37). Non-significant variation in the feed conversion ratio because of Microzist and Primalac supplementation were observed after a total period of 1st–42nd days of age (Table 4).

## *Effect of Microzist and Primalac on carcass characteristics*

Table 5 shows carcass characteristics mean of Ross 308 broilers fed diets supplemented with Microzist and Primalac from 1st to 42nd days of age. The values of carcass and eviscerated carcass were found to be increased ($P < .05$) after the supplementation of Microzist (0.2 g/kg) and Primalac into the broiler's diet. Microzist at the concentration of 0.25 g/kg showed higher breast weight (525.0 g) than that of control (447.0 g) but the weight was reduced in a comparison with the treatment containing Primalac (528.0 g). Similarly, the weights of drumstick and wings were observed to be the highest ($P < .05$) in the broiler's treatment supplemented with Microzist (0.25 g/kg) and Primalac. No significant differences in the weight of heart were observed due to Microzist and Primalac supplementation. Abdominal fat was higher in the control treatment in a comparison with lower doses of Microzist supplemented diet which showed reduction in the abdominal fat weight.

**Table 3.** Feed intake and weight gain mean of Ross 308 broilers fed diets supplemented with Microzist and Primalac from 1st to 42nd days of age (g/chick/period).

| | Feed intake mean | | | | | Weight gain mean | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment duration | Control | Microzist (0.2 g/kg) | Microzist (0.25 g/kg) | Microzist (0.3 g/kg) | Primalac (standard level) | Standard error of means (SEM) | Control | Microzist (0.2 g/kg) | Microzist (0.25 g/kg) | Microzist (0.3 g/kg) | Primalac (standard level) | Standard error of means (SEM) |
| Starter (1st–14th days of age) | 489.0$^{ab}$ | 511.0$^{ab}$ | 486.0$^{b}$ | 520.0$^{ab}$ | 542.0$^{a}$ | 15.8 | 23.11$^{a}$ | 24.02$^{a}$ | 22.97$^{a}$ | 23.47$^{a}$ | 24.92$^{a}$ | 0.99 |
| Grower (15th–28th days of age) | 1429.0$^{ab}$ | 1445.33$^{ab}$ | 1363.0$^{b}$ | 1408.0$^{ab}$ | 1526.0$^{a}$ | 45.1 | 37.97$^{ab}$ | 39.31$^{ab}$ | 37.56$^{b}$ | 39.43$^{ab}$ | 42.19$^{a}$ | 1.30 |
| Finisher (29th–42nd days of age) | 1879.0$^{b}$ | 2082.33$^{ab}$ | 1993.0$^{ab}$ | 1933.0$^{b}$ | 2177.0$^{a}$ | 66.1 | 63.68$^{a}$ | 67.45$^{a}$ | 65.62$^{a}$ | 60.64$^{a}$ | 68.95$^{a}$ | 3.04 |
| Total (1st–42nd days of age) | 3797.0$^{b}$ | 4038.70$^{ab}$ | 3842.0$^{b}$ | 3861.0$^{b}$ | 4246.0$^{a}$ | 77.5 | 47.91$^{a}$ | 50.15$^{a}$ | 48.31$^{a}$ | 47.75$^{a}$ | 52.38$^{a}$ | 1.60 |

Note: Means within each row of dietary treatments with no common superscript differ significantly at $P < .05$.

**Table 4.** Feed conversion ratio mean of Ross 308 broilers fed diets supplemented with Microzist and Primalac from 1st to 42nd days of age (g/kg).

| Treatment duration | Control | Microzist (0.2 g/kg) | Microzist (0.25 g/kg) | Microzist (0.3 g/kg) | Primalac (standard level) | Standard error of means (SEM) |
|---|---|---|---|---|---|---|
| Starter (1st–14th days of age) | 1.34[a] | 1.34[a] | 1.34[a] | 1.40[a] | 1.37[a] | 0.02 |
| Grower (15th–28th days of age) | 1.78[a] | 1.74[a] | 1.75[a] | 1.69[a] | 1.72[a] | 0.09 |
| Finisher (29th–42nd days of age) | 2.13[a] | 2.27[a] | 2.19[a] | 2.41[a] | 2.31[a] | 0.13 |
| Total (1st–42nd days of age) | 1.85[a] | 1.88[a] | 1.86[a] | 1.89[a] | 1.89[a] | 0.05 |

Note: Means within each row of dietary treatments with no common superscript differ significantly at $P < .05$.

### *Effect of Microzist and Primalac on immunological organs*

Regarding immunological organ weights, Microzist (at all concentrations) and Primalac exhibited no significant differences in the pancreas, spleen, and liver and bile weights compared to the control (Table 5).

## Discussion

Probiotics are live microbial feed additives that exhibit a beneficial impact on the health of the host and modulate the intestinal microflora, improve the meat quality of chickens, and increase the immune response. Those characteristics are due to the activation of the metabolism of one or a limited number of health-promoting bacteria, which improved the welfare of the host (Gibson and Roberfroid 1995). Different genera of probiotics such as *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have been reported to exhibit beneficial impact on broiler's performance (Ashayerizadeh et al. 2009). However, commercial probiotic preparations are used in the broiler industry in order to improve several performance factors (Talebi et al. 2008).

In the present investigation, feed intake mean of Ross 308 was found to be improved ($P < .05$) when the diet was supplemented with lower concentrations of newly developed probiotic (Microzist) and standard level of commercially available probiotic (Primalac) during a total duration of 1st–42nd days of age. The findings of the present study were in complete agreement with the reports of Pourakbari et al. (2016) and Falaki et al. (2011) who observed improved feed intake in broilers by dietary inclusion of probiotics. However, the outcomes of the present study were not found in concurrence with the reports of Jung et al. (2008) who found that the addition of probiotics did not show any significant influence on the feed intake of broiler chickens.

According to the reports of Nikpiran et al. (2013) and Nayebpor et al. (2007), probiotics supplemented treatment showed increased body weights of broilers significantly in a comparison with control group. In contrary to those studies, we estimated that Microzist and Primalac significantly lack weight gain inducing properties in broilers during a total period of 1st–42nd days of age with respect to control. Similarly, Awad et al. (2009) and Yalcinkayal et al. (2008) reported that probiotic supplementation in broilers ration had no significant effects on body weight gain. The variation in the findings of our study and previous reports might be due to the differences in the environmental conditions as well as management (nutritional constituents, structural changes in the production system, type of probiotics, humidity, light, ventilation capacity, feeding process, drinking water quality, and other physiological parameters) existing during the experiments.

Several studies had reported significant reduction in the feed conversion ratio of broilers fed diets supplemented with probiotics (Awad et al. 2009; Pourakbari et al. 2016; Sarangi et al. 2016). Our current study is against the findings of previous reports and exhibited that Microzist as well as Primalac supplemented broilers diets had no significant impact on feed conversion ratio in a comparison with control diet during a total period of 1st–42nd days of age. The outcome of the present study favoured the findings of Mutus et al. (2006) and Vargas-Rodriguez et al. (2013) who reported lack of improvement in the feed conversion ratio for the probiotic supplemented treatment. The discrepancy from some of the previous studies reported might be due to the variation in the breed and environmental conditions maintained throughout the study.

The findings of the present context were consistent with few previous reports and demonstrated that supplementing the diet with probiotic consequently improved the growth performance of broilers chicken over the whole experimental period. The improvement of overall health and growth performance of broilers could be due to the beneficial effects of

**Table 5.** Carcass characteristics and immunological organ weight mean of Ross 308 broilers fed diets supplemented with Microzist and Primalac from 1st to 42nd days of age (g).

| | Carcass characteristics | | | | | Immunological organ weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment duration | Carcass | Eviscerated carcass | Breast | Drumsticks (thighs) | Wings | Gizzard | Heart | Abdominal fat | Pancreas | Spleen | Liver and bile |
| Control | 1473.0[b] | 1223.0[b] | 447.0[b] | 348.0[b] | 102.0[b] | 55.33[a] | 9.96[a] | 12.66[ab] | 7.0[a] | 2.66[a] | 54.33[a] |
| Microzist (0.20 g/kg) | 1653.0[ab] | 1380.0[ab] | 503.0[ab] | 408.0[a] | 130.0[a] | [a]53.66 | 10.12[a] | 6.33[b] | 6.0[a] | 3.66[a] | 54.66[a] |
| Microzist (0.25 g/kg) | 1638.0[ab] | 1328.0[ab] | 525.0[a] | 380.0[ab] | 117.0[ab] | [a]63.0 | 10.47[a] | 10.0[ab] | 7.0[a] | 2.66[a] | 61.00[a] |
| Microzist (0.30 g/kg) | 1588.0[ab] | 1300.0[b] | 493.0[ab] | 350.0[b] | 117.0[ab] | [a]59.0 | 9.81[a] | 13.0[a] | 5.0[a] | 2.66[a] | 57.33[a] |
| Primalac (standard level) | 1738.0[a] | 1438.0[a] | 528.0[a] | 403.0[a] | 133.0[a] | 69.7[a] | 10.43[a] | 10.66[ab] | 6.33[a] | 3.0[a] | 57.33[a] |
| Standard error of means (SEM) | 67.4 | 39.9 | 17.8 | 16.2 | 8.19 | 4.81 | 0.31 | 1.87 | 0.59 | 0.39 | 4.51 |

Note: Means within each column of dietary treatments with no common superscript differ significantly at $P < .05$.

supplemented probiotics in the gastrointestinal tract of birds. A minor variation in the growth performances of broiler chickens from previous reports might be due to the fact that the potentiality of probiotics depends on several factors such as viability of microorganisms, mode of administration, diets composition, age of the birds, and environmental conditions (Patterson and Burkholder 2003; Wang and Gu 2010). Furthermore, the influence of broiler feed supplementation may depend on the rearing system because of the variations in the hygienic conditions (Pirgozliev et al. 2014).

Interestingly, the outcomes of the current study showed that Microzist has the unique characteristics to replace the commercially available Primalac in terms of slightly improving the growth performance of broiler chickens. In general, improvements in growth performance corresponds to the growth of health-promoting microorganisms in the gastrointestinal tract of broilers induced by dietary supplementation of Microzist in a comparison with more or less similar beneficial effect of Primalac.

The supplementation of Microzist and Primalac induced some of the carcass characteristics of broiler chickens. The weight of carcass, eviscerated carcass, breast, drumsticks, and wings was markedly improved in the broilers fed diets supplemented with Microzist and Primalac from 1$^{st}$ to 42$^{nd}$ days of age. The findings similar to our study had already been reported by Mehr et al. (2007) and Pourakbari et al. (2016) who observed an improvement in the carcass weights, breast, drumstick, and wings percentage due to the supplementation of probiotics compared to the control treatment. In contradictory, Anjum et al. (2005) and Awad et al. (2009) did not report variations in carcass percentage between a control and a probiotic supplemented treatment. Fat deposition in the abdominal region of broiler chickens is regarded as waste because it is unacceptable for consumers as well as market, and increases the cost expense during the effluent treatment. The results obtained in the current study indicate that the fat content in the abdominal region of broilers was reduced in the treatments consisting of lower dose of Microzist and Primalac. The supplementation of Microzist at higher concentration showed slight elevation in the abdominal fat content with respect to control. It had previously been observed that probiotic supplemented diets reduce the abdominal fat weight in broilers compared with the control (Anjum et al. 2005; Mehr et al. 2007; Pourakbari et al. 2016). The reduced fat content in the abdominal region of broilers might be due to the reason that reduced abdominal fat could be related to a decrease in the activity of acetyl-CoA carboxylase, the rate limiting enzyme in fatty acid synthesis, after the supplementation into basal diet (Santoso et al. 1995).

In our study, the weight of immunological organs such as pancreas and spleen in broilers fed diets supplemented with Microzist and Primalac showed no remarkable differences in comparison with control. In like manner, weights of liver and bile were observed to be unaffected due to supplementation. The findings of the current study showed complete agreement with the observations of Awad et al. (2009), Ahmadi (2011), and Naseem et al. (2012) who did not report any significant differences on the influences of probiotic supplementation on lymphoid organs.

## Conclusions

The newly developed Microzist (0.25 g/kg) and commercially available Prilamac probiotic had potentiality to improve the growth performance and carcass characteristics in broiler chickens. Microzist was observed to be an effective replacement of Primalac in order to maintain immunological organ weights. Most importantly, the inclusion of Microzist at lower concentration reduced fat content in the abdominal region of broiler chickens in a comparison with control as well as Primalac supplemented basal diet. In a nutshell, Microzist might be a promising alternative supplement in order to eliminate the application of antibiotic growth promoters in poultry industries.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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## ORCID

*Abdelfattah Z. M. Salem* http://orcid.org/0000-0001-7418-4170

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