PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF BROILER CHICKENS TO DIETARY COMBINATION OF YEAST AND MEDICAL PLANTS.

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ABSTRACT
This feeding trial was conducted to investigate the effect of medical plants and yeast cell wall on the performance and subsequent physiology of broiler chickens. A total of 200 d-old Ross 308 broiler chicks were randomly allocated to 5 treatments that were replicated 4 times of 10 birds. The first treatment was the control while the remaining four treatments were supplemented with one of these mixtures (peppermint and Chamomile; peppermint powder and yeast cell wall; chamomile powder and yeast cell wall; peppermint, yeast cell wall and chamomile powder). Throughout the experimental period, feed intake was almost not affected by dietary treatments. At days 24 and 35 of age, birds on diets containing mixtures of herbal plants or herbal plants with yeast gained more weight than the control group. Feed conversion ratio of the 24 d-old broilers was significantly improved by the combination of herbal plants and its combination with yeast. However, when assessed over the 35 d experimental period, it was almost same among all experimental units. The relative weight of visceral organs and bursal morphology were not affected by dietary treatments. Significantly lower serum cholesterol and longer jejunum villi were detected in birds that were offered diets supplemented with mixture of herbal plants alone or its combination with yeast.

Key words: medical plants, yeast cell wall, broiler performance

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INTRODUCTION

It’s well-known that broiler production is continuously facing the nutritional and health threatening problems due to the nature of its rearing. Therefore, to eliminate the stress of the intensive rearing practices and improve their well-being, broilers are continuously fortified with growth promoters and stress eliminating feeding products. Antibiotics were the most effective growth promoters that have been used in broiler nutrition. However their extensive usage has developed some health problems to the society due to its residual in the consuming poultry products and the bacterial resistance to it so far (4, 21). Therefore, the nutritional strategies to improve the growth performance of broilers while reducing its mortality via antibiotics as growth promoters have been changed after being under severe criticism in poultry nutrition (10, 16). Finding the possible natural growth promoters is becoming a must after the banning or the restriction of the use of artificial antibiotics in poultry nutrition (5). Prebiotics and medical plants have gained the interest to be used in poultry nutrition as possible alternatives to antibiotics due to their content of biological active compounds. Recently, prebiotics have been broadly supplemented into broiler diets. Prebiotics are non-digestible feed compounds; however it’s the preferable substrates that are utilized by hosts’ intestinal microbiota conferring a health benefit (8). It beneficially affects the host by stimulating the growth and the activity of bacteria in the intestinal tract, consequently improving gut health and hosts’ intestinal microbial balance. In addition dietary prebiotics have been shown to enhance digestive functionality of the poultry gastrointestinal tract (20) and positively affect animal performance by increasing body weight (7, 27) and improving feed efficiency (26). The use of medical plants as feed additives, which comprise a wide variety of herbs, has recently gained increasing interest potential alternative natural growth promoters. Among them is Chamomile flowers (Matericaria chamomilla L.), that have anti-inflammatory, antiseptic, carminative, diaphoretic, sedative properties due to its content of several numerous health benefiting phytonutrients including essential oil with azulene, bisabolo, flavonoid, glycosides and fatty acids (18, 23). Dietary chamomile positively affected the growth performance of broiler chickens (1). Peppermint (Mentha piperita L.) has also been frequently used in herbal medicine as an immune system stimulator. In addition it has antimicrobial and antioxidant and appetite enhancing characteristics (28). The benefit of dietary peppermint has been confirmed in broiler nutrition (22). This study was aimed to address the effect of the dietary combination of yeast, chamomile and peppermint on the growth performance, physiological responses and the intestinal histomorphology of broiler chickens.

MATERIALS AND METHODS

This experiment was approved by the Animal Ethic committee of the College of Agricultural Engineering Sciences, University of Duhok (Approval No: AEC 120120203). Health and animal husbandry practices complied with the Code of Practice for the Use of Animals for scientific purposes issued by the mentioned animal ethic committee.

Experimental design and bird management

In a completely randomized design, a total of 200 d-old Ross 308 broiler chicks were assigned to 5 treatments of 4 replicates of 10 birds per a replicate. Treatments were control, P + CH (basal diet supplemented with 5g/kg of each peppermint powder and Chamomile powder), P + Y (basal diet supplemented with 5g/kg of each peppermint powder and a part of Yeast Cell Wall (Catch Myco)), CH + Y (basal diet supplemented with 5gm/kg of each chamomile powder Yeast Cell Wall (Catch Myco)), P + Y + CH (basal diet supplemented with 5gm/kg of each peppermint powder, Yeast Cell Wall and chamomile powder). The main active compounds in peppermint and chamomile are presented in Table 1. All birds were vaccinated against infectious bronchitis and Newcastle diseases. Chicks were reared in floor pens (100 × 100cm) bedded with wood shaving. Three phases of feeding were adopted, a starter diet from 1 to 10 d, grower diets from 11 to 24 d, and finisher diets from 25 to 35 d. All diets were formulated to meet the requirements for Ross 308 broiler chickens (Tables 2). The room temperature was gradually decreased from 33 °C on d 1 to 24
°C ± 1 °C at 35 d. Eighteen hours of lighting were provided per d throughout the duration of the experiment, apart from d 1 to 7 when 23 hours of lighting were provided. Feed and water were provided ad libitum. On d 10, 24 and 35, the feed leftover and birds were weighed to measure the body weight, weight gain, feed intake and feed conversion ratio. Mortalities were recorded as they occurred, and feed per gain values were corrected for mortality.

Table 1. The main active compounds of peppermint and Chamomile.

| Initial concentration | DPPH Analysis (Inhibition%) | CUPRAC Analysis (Inhibition%) |
|-----------------------|-----------------------------|------------------------------|
|                       | Water Extract               | Methanol Extract             | Water Extract | Methanol Extract |
|                       | P CH                         | P CH                         | P CH          | P CH          |
| 1000 µg/ml            | 81.03                       | 84.090                      | 85.19         | 88.171        | 3.74           | 1,107         | 3.63           | 1,114         |
| 500 µg/ml             | 64.78                       | 70.529                      | 74.78         | 73.109        | 2.32           | 0.735         | 2.31           | 0.761         |
| 250 µg/ml             | 47.75                       | 65.221                      | 56.75         | 67.201        | 1.26           | 0.418         | 1.25           | 0.418         |
| 125 µg/ml             | 34.57                       | 40.964                      | 42.57         | 48.032        | 0.81           | 0.266         | 0.80           | 0.267         |
| 62.5 µg/ml            | 27.85                       | 31.628                      | 31.85         | 32.645        | 0.54           | 0.191         | 0.54           | 0.19         |

*Table entries are inhibitions (% ± SD) for DPPH and CUPRAC analyses. *Sample collection

On d 24, two birds per pen were randomly selected, weighed, and euthanized by cervical dislocation. The abdominal cavity was opened and visceral organs removed. The weights of immune related organs (liver, spleen and bursa of Fabricius) heart and gizzards were recorded and calculated as mass per unit of live body weight (g/100 of live body weight). Blood samples were collected and serum was harvested for serum biochemistry, enzymes and analyses. Approximately 1 cm of the jejunum and the whole bursa were collected for morphometric analysis. Tissue samples were opened and flushed clean with normal saline and were fixed in 10% buffered formalin for 24 hours. Formalin was subsequently replaced by 70% ethanol for long-term storage.

Serum biochemical parameters

Blood samples (approximately 5 ml) were collected from the jugular vein into non-heparinized tubes. Subsequently, serum was harvested after centrifuging the blood for 15 min and stored in the refrigerator for analyses. Serum biochemical parameters including total protein, albumin, globulin, cholesterol, alanine transaminase (ALT) and aspartate aminotransferase (AST) were determined using an automatic COBAS INTEGRA400 plus analyzer (Cedex Bio HT Analyzer).

Jejunum and bursa histology

Tissue samples were collected and fixed in 10% neutral buffered formalin for histomorphological analysis. Samples were embedded in paraffin wax, sectioned and stained with haematoxylin and eosin. Sample sections were captured at 10× magnification using a digital camera under microscope (Dino-Eye-Microscope Eye-piece Camera) and morphometric indices were determined by Dino-eye program. Images were digitized and the villus height (from the tip of the villus to the villus/crypt junction), crypt depth (from the villus/crypt junction to the muscular junction) and jejunal muscular thickness were measured in 10 well-oriented villi for each jejunal section. The apparent villus surface area was also calculated using the formula: \( [(\text{villus tip} + \text{villus base})/2] \times \text{villus height} \) (13). The length and width of 10 Bursal follicles and its area were measured by the same technique.
Table 2. Ingredients and nutrient composition of starter, grower and finisher diets as percentage.

| Ingredients kg | Starter | Grower | Finisher |
|---------------|---------|--------|----------|
| Corn          | 53.06   | 56.9   | 61.74    |
| Soybean meal  | 31.48   | 32.89  | 28.23    |
| Fish meal     | 4       | -      | -        |
| Vegetable oil | 3       | 4.48   | 4.57     |
| Limestone     | 2       | 1.39   | 1.35     |
| Dical Phos    | 2.72    | 0.95   | 0.82     |
| Salt          | 0.11    | 0.19   | 0.08     |
| D.L-methionine| 0.38    | 0.32   | 0.24     |
| L-lysine HCl  | 0.5     | 0.26   | 0.21     |
| L-threonine   | 0.25    | 0.13   | 0.09     |
| Broiler premix| 2.5     | 2.5    | 2.5      |

Nutrient composition

| ME (kcal/kg) | 3000 | 3150 | 3200 |
| Crude protein| 23   | 21   | 19.16 |
| Crude fiber  | 2.25 | 2.38 | 2.33  |
| Digestible Arginine | 1.29 | 1.14 | 0.99 |
| Digestible Lysine     | 1.29 | 1.14 | 0.99 |
| Digestible Meth+Cyst  | 0.87 | 0.84 | 0.73 |
| Digestible Tryptophan | 0.226 | 0.24 | 0.21 |
| Digestible Isoleucine | 0.87 | 0.81 | 0.73 |
| Digestible Threonine | 0.82 | 0.73 | 0.63 |
| Digestible Valine    | 0.99 | 0.92 | 0.83 |
| Calcium               | 1.60 | 0.9  | 0.85 |
| Available Phosphorus | 0.844 | 0.450 | 0.42 |
| Sodium                | 0.16 | 0.160 | 0.16 |
| Chloride              | 0.35 | 0.312 | 0.23 |
| Linoleic              | 2.18 | 2.64 | 2.73 |

Statistical analysis of data
In a complete randomize analysis, all collected data were analyzed by one way ANOVA of SAS 2013. Differences between mean values were determined using Duncan’s multiple range test.

RESULTS AND DISCUSSION

Growth performance
At day 10 of birds’ age, Feed intake was not affected by dietary treatments except for those on diets containing a mixture of Peppermint + Yeast cell wll that were consumed less feed than other experimental groups (Table 3). However, feed consumption was almost same in all experimental groups in the subsequent grower and finisher periods. Body weight gain of broilers was not affected by treatments in the first 10 days of their age. However, over the 24 days of broilers’ age, it significantly increased in all experimental groups compared to the control group. When assessed over the 35 days experimental period, body weight gain was improved in all additive supplemented groups compared to the control groups. Feed conversion ratio was not affected by dietary treatments in the first 10 days of birds’ age. However at day 24, it significantly improved by dietary yeast, peppermint and chamomile supplemented birds. Feed conversion ratio of all groups was almost same over 35 days of broilers age. The results of the current study demonstrated that broiler performance was improved by dietary herbal plants and yeast. The positive effect of feeding herbal plant and its by-products on the performance and the productivity of broilers has been confirmed. This could be attributed to the presence of various active compounds that could enhance feed consumption, improve the secretion and activity of digestive enzymes and increase the intestinal digestion and absorption of nutrients (19). In addition, its ability to eliminate the pathogenic bacteria in the gut, medical plants may decrease the degradation of protein and amino acid and thus improve the absorption and accretion in the body and improve the carcass weight (17). Administration of dietary prebiotics has been demonstrated to promote populations of beneficial bacteria and decrease populations of pathogens in the GI tract in poultry (24), and prebiotics have been suggested as potential alternatives to AGP because of their ability to improve growth performance similarly to antibiotics (11).
was in line with finding of (16) when different levels of chamomile were fed to broilers. Abdel-Wareth et al. (2) reported that broilers body weight and weight gain were significantly increased by dietary peppermint. Furthermore, (9, 21) found that incorporation of peppermint to the diet significantly improved the body weight, weight gain and feed conversion ratio of broiler chickens. Froebel et al. (7) found that dietary peppermint significantly increased by dietary peppermint. Therefore, we hypothesized that 1:52 (5:20) of the mixture of medical plants and yeast cell wall on the internal organs may be functional not physical.

### Table 3. Effect of different treatments on the broiler performance

| Period | Treatments  | Control | P+CH | P+Y | CH+Y | P+Y+CH | Pooled SEM | P-Value |
|--------|-------------|---------|------|-----|------|--------|------------|---------|
|        | Feed intake (g/bird) |         |      |     |      |        |            |         |
| 1-10d  | 295.5<sup>a</sup> | 297.0<sup>a</sup> | 259.0<sup>b</sup> | 291.5<sup>a</sup> | 295.5<sup>a</sup> | 4.168     | 0.003     |
| 1-24d  | 1369.0      | 1385.0  | 1367.5 | 1380.8 | 1337.3 | 8.204     | 0.407     |
| 1-35d  | 2794.3      | 2935.5  | 2967.5 | 2935.8 | 2903.3 | 25.159    | 0.224     |
|        | Body weight gain (g/bird) |        |      |     |      |        |            |         |
| 1-10d  | 247.8       | 255.8   | 228.3 | 251.5 | 244.5 | 3.968     | 0.230     |
| 1-24d  | 982.3<sup>b</sup> | 1074.0<sup>a</sup> | 1118.5<sup>a</sup> | 1105.0<sup>a</sup> | 1037.8<sup>b</sup> | 13.511    | 0.001     |
| 1-35d  | 1894.8      | 1985.5  | 2013.5 | 1988.8 | 1967.8 | 17.001    | 0.230     |
|        | Feed conversion ratio (FCR) |        |      |     |      |        |            |         |
| 1-10d  | 1.199       | 1.165   | 1.138 | 1.161 | 1.211 | 0.013     | 0.440     |
| 1-24d  | 1.394<sup>c</sup> | 1.290<sup>b</sup> | 1.222<sup>c</sup> | 1.252<sup>bc</sup> | 1.289<sup>c</sup> | 0.015     | <0.001    |
| 1-35d  | 1.475       | 1.479   | 1.474 | 1.476 | 1.476 | 0.003     | 0.993     |

*<sup>a</sup>-<sup>c</sup> means values on the same rows not sharing a superscript are significantly different. Control = Basal diet + No additive. P+CH = Peppermint + Chamomile. P+Y = Peppermint + Yeast. CH+Y = Chamomile + Yeast. P+Y+CH = Peppermint + Yeast + Chamomile.

### Serum biochemistry

Among the measured serum biochemical parameters, the cholesterol was decreased in birds that were fed on diets containing the mixture of peppermint and chamomile followed by those offered diets that contained the mixture of peppermint, chamomile and yeast (Table 5). The higher cholesterol level in the serum was recorded on birds that consumed the diet that was supplemented by the mixture of chamomile and yeast compared to the other experimental groups. The combination of medical plants and yeast cell wall in the current study had hypocholesterolic effect. Lipase activity of both pancreas and gastric origin could be reduced by dietary herbal plants, thus reducing the fat digestibility in the gastrointestinal tract (6). Furthermore, herbal plants may inhibit fat metabolism thereby interfering with the cholesterol solubility in the gastrointestinal tract decreasing its absorption and increasing the excretion of bile acid in the feces (29). The hypocholesterolic effect of chamomile was also reported by (3, 16).

### Table 4. Effect of different treatments on the relative internal organs weights (g/100g BW) of broilers at d 24 of age.

| Treatments<sup>a</sup> | Liver | Heart | Spleen | Gizzard | Small Intestine | Bursa of Fabricius |
|------------------------|-------|-------|--------|---------|-----------------|------------------|
| Control                | 3.11  | 0.777 | 0.104  | 3.726   | 7.078           | 0.147            |
| P+CH                   | 3.06  | 0.600 | 0.077  | 4.209   | 7.055           | 0.151            |
| P+Y                   | 4.04  | 1.520 | 0.169  | 5.561   | 12.089          | 0.368            |
| CH+Y                  | 2.72  | 0.580 | 0.091  | 5.250   | 6.771           | 0.175            |
| P+Y+CH                | 2.76  | 0.578 | 0.090  | 3.696   | 7.496           | 0.155            |
| Pooled SEM            | 0.205 | 0.132 | 0.016  | 0.294   | 0.719           | 0.033            |
| P-Value                | 0.247 | 0.089 | 0.419  | 0.120   | 0.078           | 0.154            |

<sup>a</sup>Control = Basal diet + No additive. P+CH = Peppermint + Chamomile. P+Y = Peppermint + Yeast. CH+Y = Chamomile + Yeast. P+Y+CH = Peppermint + Yeast + Chamomile.
Table 5. Effect of different treatments on serum biochemical parameters of broilers at d 24 of age.

| Treatments* | Cholesterol (mg/dl) | ALT (ul) | AST (ul) | Total Protein (g/dl) | Albumen (g/dl) | Globulin (g/dl) |
|-------------|---------------------|----------|----------|----------------------|---------------|---------------|
| Control     | 91<sup>a,b</sup>    | 2.43     | 158      | 2.533                | 1.058         | 1.475         |
| P+CH        | 78<sup>b</sup>      | 1.93     | 146      | 2.178                | 0.838         | 1.340         |
| P+Y         | 98<sup>a,b</sup>    | 1.73     | 164      | 2.663                | 1.010         | 1.653         |
| CH+Y        | 107<sup>a</sup>     | 1.95     | 174      | 2.598                | 1.035         | 1.563         |
| P+Y+CH      | 80<sup>b</sup>      | 2.45     | 130      | 1.836                | 0.766         | 1.069         |
| Pooled SEM  | 3.627               | 0.129    | 5.900    | 0.121                | 0.047         | 0.077         |
| P-Value     | 0.0399              | 0.2914   | 0.1543   | 0.1475               | 0.1882        | 0.1226        |

<sup>a,b</sup> Mean values on the same column not sharing a superscript are significantly different at (P < 0.05).

*Control = Basal diet + No additive, P+CH = Peppermint + Chamomile, P+Y = Peppermint + Yeast, CH+Y = Chamomile + Yeast, P+Y+CH = Peppermint + Yeast + Chamomile; ALT = Alanine transaminase, and AST = Aspartate aminotransferase (AST).

Jejunum and bursal histomorphology

Villi were significantly longer in all experimental groups compared to the control (Table 6). Crypt depth significantly decreased in birds that were fed diets containing the mixture of peppermint and yeast cell wall compared to the control group followed by those on diets supplemented with the mixture of chamomile and yeast. Villi height to crypt depth ratio increased by dietary treatment, however it was only significant in birds that were fed diets contained the mixture of peppermint and yeast compared to the control group. Villi tip width, base width and the apparent villi surface area were significantly decreased in all experimental groups compared to the control. Jejunum muscle thickness increased in birds on diets containing the mixture of chamomile and yeast cell wall, however that of birds on diets containing the mixture of peppermint and yeast was significantly the less compared to the control and other experimental groups. In this study there was an improvement in the jejunal histomorphology of broiler chickens due to the consumption of a mixture of chamomile, peppermint and yeast cell wall. This may be attributed to the presence of bioactive compounds in the medical plants that could improve the gut health and intestinal morphology. Improving the jejunal morphology of birds including villus height or villus height-to-crypt depth ratio may point out to the efficient digestion, better absorptive capacity which may lead to the better nutrient uptake and utilization and finally better performance. These findings were in line with those of (15, 25) when phytogenic additives were included in the broiler diets. Similar findings have also been found by (9) when peppermint was included to the broiler diets. Furthermore (16) have also obtained the similar findings by dietary chamomile. In addition to the effect of medical plants, yeast cell wall may also involve in the development of the intestinal morphology thereby enhancing the production of short chain organic acids that may induce as a result of the fermentation of dietary prebiotic by the bacterial community in the intestine. The short chain organic acids which are by products of bacterial fermentation stimulate the proliferation of epithelial cells of the bowel (12). In addition, some bacteria may recognize binding sites on the prebiotic as if they were from the intestinal mucosa and the colonization of the intestine by pathogenic bacteria is thus reduced, therefore besides a lower infection incidence; there is an increase in the absorption of available nutrients, a mechanism that directly affects the recovery of intestinal mucosa, increasing villi height (14).
Table 6. Effect of different treatments on jejunum muscle thickness, villus height, crypt depth and apparent villus surface of broilers at d 24 of age.

| Treatments | Villus height μm VH | Crypt depth μm CD | VH/CD ratio | villus tip width μm | villus base width μm | apparent villus surface area μm² | Jejunum muscle thickness μm |
|------------|---------------------|------------------|-------------|---------------------|---------------------|-------------------------------|----------------------------|
| Control    | 945<sup>a</sup>     | 183<sup>a</sup>  | 5.55<sup>a</sup>  | 241<sup>a</sup> | 247<sup>a</sup>  | 230151<sup>a</sup> | 204<sup>a</sup>             |
| P+CH       | 1093<sup>b</sup>    | 168<sup>b</sup>  | 6.86<sup>b</sup>  | 137<sup>b</sup> | 140<sup>b</sup>  | 149719<sup>b</sup> | 200<sup>b</sup>             |
| P+Y        | 1044<sup>b</sup>    | 146<sup>b</sup>  | 7.28<sup>a</sup>  | 141<sup>b</sup> | 141<sup>b</sup>  | 147856<sup>b</sup> | 142<sup>c</sup>             |
| CH+Y       | 1015<sup>b</sup>    | 191<sup>c</sup>  | 5.47<sup>b</sup>  | 152<sup>b</sup> | 172<sup>b</sup>  | 164158<sup>b</sup> | 258<sup>b</sup>             |
| P+Y+CH     | 1011<sup>c</sup>    | 166<sup>b</sup>  | 6.46<sup>b</sup>  | 163<sup>b</sup> | 182<sup>b</sup>  | 174911<sup>b</sup> | 201<sup>b</sup>             |
| Pooled SEM | 9.43                | 4.31             | 0.20         | 7.08               | 7.44               | 5989             | 5.66                      |
| P-Value    | <.0001              | 0.0209           | 0.0295       | 0.0001             | <.0001             | 0.0083          | <.0001                    |

<sup>a,b,c</sup> = Mean values on the same column not sharing a superscript differ significantly different at (P < 0.05).

*Control = Basal diet + No additive. P+CH = Peppermint + Chamomile. P+Y = Peppermint + Yeast. CH+Y = Chamomile + Yeast. P+Y+CH = Peppermint + Yeast + Chamomile

Bursa histomorphological parameters including (bursa follicles length, bursa follicles width and bursa lymphoid follicles areas) were almost same in all experimental groups (Table 7). This may due to that these birds were reared in a sanitary condition. The effect of medical plants and yeast cell wall could be more pronounced when birds are exposed to disease challenges.

Table 7. Effect of different treatments on morphometric cloacal bursa measurements of broilers at d 24 of age.

| Treatments | Bursa follicles length μm | Bursa follicles width μm | Bursa Lymphoid follicles area μm² |
|------------|---------------------------|--------------------------|-----------------------------------|
| Control    | 5018                      | 2985                     | 12909                             |
| P+CH       | 5220                      | 2508                     | 10296                             |
| P+Y        | 3950                      | 2537                     | 8340                              |
| CH+Y       | 4539                      | 2900                     | 10668                             |
| P+Y+CH     | 4810                      | 2808                     | 10938                             |
| Pooled SEM | 169.6                     | 126.3                    | 822.6                             |
| P-Value    | 0.1359                    | 0.7212                   | 0.5766                            |

*Control = Basal diet + No additive. P+CH = Peppermint + Chamomile. P+Y = Peppermint + Yeast. CH+Y = Chamomile + Yeast. P+Y+CH = Peppermint + Yeast + Chamomile

CONCLUSION

This study demonstrated that the combination of different medical plants and their incorporation with the yeast cell wall were effective in improving the broiler performance. The hypocholesterolemic effect was clear in birds by dietary mixture of medical plants or their mixture with yeast cell wall. The dietary supplementation of medical plants and their mixture with yeast cell wall had a positive effect on the intestinal morphology of broiler chickens. This was confirmed by longer villi and higher villi height/crypt depth in the medical plants and yeast cell wall supplemented birds. A cocktail of medical plants and their combination with yeast cell wall could be more effective in broiler production.

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