Effects of a yeast cell wall product on the performance of broiler chickens and PGC-1α, TLR4, IL-10 and PPARγ genes expression

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ABSTRACT

The current study was conducted to evaluate the effects of a yeast cell wall product (YCWP) on the performance, the population of *Lactobacillus* and coliform bacteria and PGC-1α, TLR4, IL-10 and PPARγ genes expression in the intestine of broilers. Dietary treatments were as follows: basal diet (control), control diet + 0.3 g/kg colistin or 0.15, 0.3, 0.45 and 0.6 g/kg YCWP. Feed intake and feed conversion ratio were lower in the YCWP-fed birds compared to the control. Colistin and YCWP fed birds had higher *Lactobacillus* and lower coliform bacteria counts than control birds (*p* < .05). Total and IgM antibody against sheep red blood cells was linearly increased by adding the YCWP to the diet (*p* < .05). Adding the YCWP to the diet linearly increased the caecal concentration of propionate (*p* < .05). Orthogonal and polynomial contrasts showed that the YCWP significantly improved the villus height, crypt depth, villus height: crypt depth, villus width, and villus surface area (*p* < .05). The lower concentrations of the YCWP (0.15 and 0.30 g/kg) increased the PPARγ transcription (*p* < .05), while its upper concentration (0.45 and 0.6 g/kg of diet) did not change the PPARγ gene expression. PGC-1α transcription was increased with 0.15 g/kg of the YCWP supplementation (*p* < .05). Dietary inclusion of the YCWP decreased the TLR4 gene expression (*p* < .05) and increased the downstream IL-10 cytokine (*p* < .05). In conclusion, YCWP improved the broiler’s performance by modifying the gut microflora. Also, YCWP supplementation had an immune regulatory effect via local and systemic responses involving TLR4, IL-10, and PPARγ.

HIGHLIGHTS

- Adding YCWP to the broilers diet did not significantly affect body weight gain, but reduced FCR.
- Adding 0.45 and 0.6 g of YCWP to the diet, increased the *Lactobacillus* number and reduced the coliform bacteria number in the ileum.
- YCWP supplementation increased the production of antibodies (total, IgG, and IgM) against SRBC and developed lymphatic organs.
- Adding 0.6 g of YCWP to the diet increased villus height and villus surface area and reduced the crypt depth.
- YCWP supplement had an immune regulatory effect via local and systemic responses involving TLR4, IL-10, and PPARγ.

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Introduction

During the last 30 years, scientists have attempted to develop strategies to enhance livestock production, reduce production costs and mitigate the harmful environmental effects. The increase in pathogenic bacteria in the birds’ gastrointestinal tract (GIT) could impair growth and feed utilisation. Such pathogenic bacteria may attach to the various parts of the GIT in the poultry, causing considerable damage to the epithelial layer cells (Vermeulen et al. 2001; Ritzi et al. 2016). Therefore, there is an immediate need to develop new strategies that can sufficiently protect broiler chickens against the population of harmful bacteria without detrimental effects on the broilers’ performance (Ott et al. 2018). Alternative additives to antibiotics are being sought to provide a helpful product for animal production due to the ban and strict standards in using antibiotics. As such, the poultry industry is under immense pressure to decrease the...
use of antibiotics in commercial production, leading to more research to find the compounds capable of improving the GIT health and immune function in the poultry (Ott et al. 2018).

Today, the use of prebiotics in poultry feeds is widely considered to increase the bird’s performance. Prebiotics are defined as the indigestible feeds that can stimulate the growth of one or a limited number of bacteria in the gut, with positive effects on the host (Gadde et al. 2017). The most common prebiotics used in poultry feeds are yeast cell wall products (YCWP) such as inulin, fructooligosaccharides (FOSs), mannan-oligosaccharides (MOSs), β-glucan, xylooligosaccharides, and galactooligosaccharides (GOSs) (Ricke 2018). For example, MOSs are oligomers made of mannose linked together by β-1, 4 glycoside bonds; they are found in the cell wall of Saccharomyces cerevisiae (Pourabedin et al. 2014). Mannan-oligosaccharides are usually derived from yeast cells, which modulate the immune system and eliminate pathogenic bacteria from the GIT. Mannans present in MOSs can bind to mannose-specific type-I fimbriae in gram-negative bacteria (such as E. coli, Salmonella and Clostridium), preventing them from attaching and colonising the intestinal epithelium (Suresh et al. 2018).

To find out how YCWP is working on the surface of the GIT cells, the present study investigated the expression of genes that are possibly involved in the regulation of metabolism and reaction to the pathogenic bacteria and lipopolysaccharides (LPS) in the epithelial cells under the YCWP treatment. Peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) is a transcriptional coactivator characterised as a regulator of mitochondrial biogenesis and function, including oxidative phosphorylation and detoxification of the reactive oxygen species. PGC-1α is highly expressed in the tissues with high-energy demands (Rius-Pérez et al. 2020). PGC-1α also regulates the expression of mitochondrial antioxidant genes, which alleviate oxidative injuries and mitochondrial dysfunctions. Dysregulation of PGC-1α exacerbates the inflammatory response, which is commonly accompanied by a metabolic disorder. Following the inflammation, the small levels of PGC-1α downregulate mitochondrial antioxidant gene expression, persuade oxidative stress and promote the nuclear factor kappa B activation. Toll-like receptors (TLRs) is one of the vital plasma membrane receptors that could create connections between luminal pathogens and genes expression of intestinal epithelial cells involved in metabolism such as PGC-1α. TLRs are a class of proteins that play a crucial role in the innate immune system, serving as the first line of the host’s response against besetting microbial pathogens (Luo et al. 2014). Various factors can affect TLRs expression and modulate TLRs signalling pathways, leading to the release of endogenous molecules serving as TLR ligands and the responsiveness of innate and adaptive immune systems to combat pathogenic bacteria (Arslan et al. 2011; Napper et al. 2015; Paul et al. 2015). Previous studies demonstrated that TLR4 transmits LPS signals (Yang et al. 1998). However, rearing conditions could modulate the inhibition of the GIT microflora of broilers for the recognition of pathogen-associated molecular patterns to occur by TLRs, enabling the activation of the immune cells in the GIT (Li et al. 2020). Besides, resistance to the pathogens has been shown to be closely correlated with the up-regulation of TLR4, as well as various chemokines and cytokines (Henri et al. 2020; Takizawa et al. 2020). Therefore, PGC-1α acts as a necessary factor connecting metabolic modifications and inflammatory pathways. It is, therefore, an attractive target gene that has considerable benefits for the regulation of the GIT homeostasis under the effect of bacterial populations (Rius-Pérez et al. 2020). There is, however, limited information regarding the effects of YCWP on the expression of genes involved in the immune responses of broiler chickens, including peroxisome proliferator-activated receptor γ (PPARγ), PGC1α, TLR4 and interleukin-10 (IL-10). Based on the previous studies, we hypothesised that the effects of YCWP on the immune system of the broiler chickens could be mediated by regulating the gene expression involved in inflammation and mitochondrial energy metabolism. Therefore, this study aimed to evaluate the effects of adding a YCWP to the broiler diets on the performance, the population of Lactobacillus and coliform bacteria in the ileum, morphology of jejunum, immune system and PPARγ, PGC1α, TLR4 and IL-10 genes expression, which have not been previously explored.

Materials and methods

Birds management and experimental design

This experiment was conducted according to the comprehensive guide of animal welfare, as adopted by FASS (2010). The Animal Policy and Welfare Committee of the Isfahan University of Technology approved all animal care and experimental procedures. This experiment was conducted on a broiler research farm. This broiler research house was equipped to optimise the environmental condition and provide the best animal welfare during the experimental period.
The uniform environmental conditions between all experimental units (floor pen) provided by synchronising of heating systems (floor heating system and warm air tunnels), sidewall and tunnel fans, cooling systems (the cooling pads were located behind sidewall inlets), fogging system (one nozzle was situated in front of each sidewall inlet) and controlled through a computerised ventilation system controller. The temperature was maintained at 33°C for the first two days and then reduced gradually to 22°C until 39 days of age. The light regime was 23 L: 1 D for the first three days and then 20 L: 4 D till the end of the experiment. Chickens were fed ad libitum with the standard mash diets (based on Ross 308 recommendation, Table 1) during the starter (1–10 d), grower (11–24 d), and finisher phases (25–39 d).

A total of 1440 day-old as-hatch broilers (Ross 308) with an average weight of 37.35 ± 0.82 g were used in a completely randomised design with six treatments and ten replicates of 24 chickens. The treatment groups included corn-soy based diet (control), control + 0.15 g/kg of YCWP, control + 0.3 g/kg of YCWP, control + 0.45 g/kg of YCWP, control + 0.6 g/kg of YCWP, and control + 0.3 g/kg of colistin antibiotic. In this experiment, YeaMune-UP® (Pathway Intermediates Company, Seoul, South Korea) was used as a YCWP supplement. The YeaMune-Up is a functional fermented product made from Saccharomyces cerevisiae boulardii; it is rich in β-glucan and mannan-oligosaccharides (MOS). The body weight of each bird was recorded individually on days 10, 24, and 39 of age after 2 hours of fasting. Feed intake was recorded at 10, 24, and 39 d of age. The average daily weight gain, average daily feed intake, feed conversion ratio (FCR), and European efficiency factor (EEF) were calculated accordingly. Also, at 40 days of age, one male bird from each replicate was selected and humanely euthanized by CO2 for samples collection.

### Table 1. The composition and nutrient content of the control diet (g/Kg, as-fed basis).

| Ingredients                  | Starter         | Grower         | Finisher        |
|------------------------------|-----------------|----------------|-----------------|
| Corn                         | 518.2           | 563.7          | 624.6           |
| Soybean meal (CP 42%)        | 370.0           | 349.0          | 281.0           |
| Soybean oil                  | 20.0            | 25.5           | 30.3            |
| Corn gluten meal (CP 60%)    | 50.0            | 25.0           | 30.0            |
| Salt                         | 2.1             | 2.2            | 1.9             |
| NaHCO3                       | 2.3             | 2.2            | 2.6             |
| Di-calcium phosphate         | 16.5            | 13.8           | 12.2            |
| Limestone                    | 10.4            | 9.7            | 8.7             |
| Vitamin premixa              | 1.0             | 1.0            | 1.0             |
| Mineral premixb              | 1.0             | 1.0            | 1.0             |
| L-lysine-HCL                  | 3.4             | 2.5            | 2.7             |
| DL-methionine                | 3.0             | 2.8            | 2.6             |
| L-threonine                  | 1.0             | 0.7            | 0.5             |
| Choline chloride             | 1.0             | 0.8            | 0.8             |
| Phytase 5000 (FTU/g)         | 0.1             | 0.1            | 0.1             |

Analysed composition

| Metabolisable energy (Kcal/Kg) | 2985.0 | 3040.0 | 3155.0 |
| Crude protein (%)              | 23.0   | 20.90  | 18.82  |
| Digestible lysine (%)          | 1.28   | 1.15   | 1.02   |
| Digestible methionine (%)      | 0.64   | 0.58   | 0.54   |
| Digestible methionine + cysteine (%) | 0.95 | 0.87 | 0.80 |
| Digestible threonine (%)       | 0.86   | 0.77   | 0.68   |
| Calcium (%)                    | 0.96   | 0.87   | 0.78   |
| Available phosphorus (%)       | 0.48   | 0.43   | 0.39   |

*Supplied per kg of diet: 12,000 U Vit A, 5000 U Vit D₃, 80 IU Vit E, 3.2 mg Vit K, 3.2 mg Vit B₁, 8.6 mg Vit B₂, 65 mg niacin, 20 mg pantothenic acid, 4.3 mg Vit B₆, 0.22 mg biotin, 2.2 mg folic acid, 0.017 mg Vit B₁₂.

*Supplied per kg of diet: 16 mg copper, 1.25 mg iodine, 20 mg iron, 120 mg manganese, 0.3 mg selenium, 110 mg zinc.

*One FTU is the quantity of enzyme that releases 1 μmol of inorganic P/min from 0.00001 mol/L sodium phosphate at pH 5.5 at 37°C.

### Ileal sample collection for detecting the Lactobacillus and Coliform bacteria

Ileal contents of six chickens from each treatment were used to enumerate *Lactobacillus* and coliform bacteria at the end of the experiment. One g of digesta was collected (from the last 10 cm of the ileum) and mixed with 9 mL of peptone water broth; 100 μL of the solution was then homogenised in 900 μL phosphate-buffered saline (PBS) and serially (1:10) diluted. The population of *Lactobacillus* was counted on the De Man, Rogosa, and Sharpe (MRS) agar (Man, Rogosa, and Sharpe agar) and incubated in an anaerobic incubator at 37°C for 48 hours. The coliform bacteria were also counted on the MacConkey agar and incubated at 37°C for 24 hours. The results were expressed as log10 of colony-forming units (CFU) per gram of ileal digesta.

### Analysis of cecal short chain fatty acids (SCFAs)

To measure the concentration of short-chain fatty acids in the caecum, ten birds from each treatment were chosen randomly at the age of 40 days; the caecum contents for each were collected and stored at −20°C. One g of caecum contents was suspended in 4 mL of distilled water in a sterile tube. The samples were homogenised and centrifuged at 4,000 × g for 15 min. One mL of the supernatant was then transferred into an Eppendorf tube and mixed with 250 μL metaphosphoric acid solution. The solutions were then centrifuged again at 4,000 × g for 15 min. After that, 1 mL of the supernatant was transferred into an Eppendorf tube mixed with 200 μL crotonic acid solution as the internal standard. The solutions were centrifuged again at 4,000 × g for 15 min. The supernatant was analysed by gas chromatography (GC) for the
concentrations of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate (Zhang et al. 2003).

**Analysis of the immune system response**

**Weighing the lymphatic organs**
At the end of the experiment, lymphoid organs such as the bursa of Fabricius, thymus, and spleen were separated and weighed individually from ten euthanized chickens per treatment. The relative lymphoid organs weight was calculated and presented as a percentage of live body weight.

**Anti-SRBC antibody assay**
Ten birds from each treatment were injected with 0.5 mL of 5% suspension of sheep red blood cell (SRBC) on days 22 and 29 of age to evaluate the antibody production potential (Seidavi et al. 2017). Blood samples were collected 7 and 14 days after the first injection. The serum of each sample was isolated and frozen at −20°C. Anti-SRBC antibody was measured using the microhemagglutination assay in 96 well microplates, as described by Akhlaghi et al. (Akhlaghi et al. 2013).

**Intestinal morphological analysis**
For enteric morphology analysis, at the end of the experiment, the jejunal samples were collected. One cm of the jejunal’s midpoint from each bird was removed and fixed in 10% buffered formalin. Samples were prepared as described by Ekim (Ekim et al. 2020) for evaluation by optical microscope (Olympus CX31, Tokyo, Japan) and photographed with a digital microscope camera. Crypt depth, height and width of villi, and muscular layers thickness were measured using ImageJ software (Sakamoto et al. 2000; Ekim et al. 2020). The formula for calculating the villus surface area was $2\pi \times \text{villus width/2} \times \text{villus height}$ (Prakatur et al. 2019).

**Samples preparation and total RNA extraction**
At the age of 40 days, 2 cm of the ileum of 3 chickens from each treatment was obtained for gene expression analysis. The collected tissue samples were washed with distilled water and immediately frozen in liquid nitrogen and stored at −80°C until further use. Total RNA was isolated from the intestine tissue samples of the broilers separately using TRIzol (Sinaclon, Tehran, Iran), according to the manufacturer’s protocol; RNase-free DNasel was used to remove DNA contamination. Dried RNA samples were dissolved in DEPC water. RNA integrity was verified by agarose gel electrophoresis and visualisation of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression RNA. The concentration of the RNA samples was determined using Nano Drop-2000 (Thermo, USA).

**Real-time quantitative RT-PCR (qRT-PCR) analysis**
The expression of four candidate genes, including PPARγ, PGC-1α, TLR4, and IL-10, was determined using quantitative reverse transcription-PCR (qRT-PCR, ABI StepOne™ Real-Time PCR System – Thermo Fisher Scientific). The reaction was performed using RealQ Plus 2x Master Mix Green (Amplicon). Primers were designed based on the target genes sequences and blasted with the NCBI Blast Primer; then, they were synthesised commercially (TAG, Copenhagen, Denmark, Table 2). The primers are listed in Table 2. Complementary DNA (cDNA) was synthesised from the total RNA using cDNA synthesis® RT reagent Kit (Sinaclon). For each sample, 25 ng of cDNA was used as a template in the final volume of 25 μL reaction. The thermal cycling conditions had an initial denaturation step at 95 °C for 10 min; this was followed by 40 cycles, including a denaturation step at 95 °C for the 30 s, and the annealing and extension step at 60 °C for 30 s. The GAPDH gene was used as an internal control. Each experiment was performed in triplicate and repeated three times independently. The triplicate PCRs’ cycle threshold (Ct) values were averaged, and the relative quantification of the transcript levels was performed using the comparative 2−ΔΔCt method. The fold change in the target gene, relative to GAPDH, was

| Gene     | Forward primer (5'-3') | Reverse primer (5'-3') | Reference               |
|----------|------------------------|------------------------|-------------------------|
| GAPDH    | GAAGCTTACTGGAATGCTTTCC | CCGCAGGTAGTCAACAA      | Khan et al. 2017        |
| PPARγ    | CACTGAGAAGACAAAGAAGAA  | TCCACAGGCAAACGTGATC    | Wang et al. 2017        |
| PGC1-α   | GACACAACACGGACAGAAT    | GCATCAGGGTATAACGTTAAGG | Baghi et al. 2020       |
| TLR4     | AGTCTGAAAATGGCTAGCTAAT | GCGACCTAAGCCGGAAG      | Asgari et al. 2018      |
| IL-10    | CTGTCACCGCTTCCTCACC   | ACTCCCCCATGGTCTTGTGA   | Lee et al. 2021         |

GAPDH: glyceraldehyde 3-phosphate dehydrogenase; PPARγ: peroxisome proliferator-activated receptor γ; TLR4: Toll-like receptor 4; IL-10: Interleukin-10.
Table 3. Effect of adding yeast cell wall product (YCWP) to the diet on performance during experimental periods.

| Item         | Control | 0.15 g YCWP | 0.3 g YCWP | 0.45 g YCWP | 0.6 g YCWP | Colistin | SEM | Trt | C vs. YCWP | Lin | Quad |
|--------------|---------|-------------|------------|-------------|------------|----------|-----|-----|------------|-----|-------|
| 0–10 Day     |         |             |            |             |            |          |     |     |            |     |       |
| ADWG<sub>g</sub>, g | 21.20   | 21.36       | 21.06      | 21.42       | 21.08      | 21.58    | 0.309 | 0.812 | 0.932      | 0.856 | 0.773 |
| ADFI<sub>e</sub>, g  | 24.56   | 24.95       | 24.44      | 24.39       | 24.33      | 24.26    | 0.345 | 0.652 | 0.812      | 0.218 | 0.669 |
| FCR<sub>f</sub>   | 1.16    | 1.16        | 1.16       | 1.14        | 1.15       | 1.12     | 0.022 | 0.664 | 0.655      | 0.384 | 0.983 |
| 11–24 Day    |         |             |            |             |            |          |     |     |            |     |       |
| ADWG<sub>g</sub>, g | 52.73   | 52.84       | 52.55      | 52.26       | 52.28      | 50.92    | 0.710 | 0.448 | 0.736      | 0.519 | 0.942 |
| ADFI<sub>g</sub>, g  | 70.98   | 70.22       | 68.05      | 67.53       | 67.70      | 68.40    | 1.204 | 0.230 | 0.068      | 0.023 | 0.456 |
| FCR<sub>e</sub>   | 1.35    | 1.33        | 1.29       | 1.29        | 1.29       | 1.29     | 0.017 | 0.090 | 0.049      | 0.027 | 0.342 |
| 25–39 Day    |         |             |            |             |            |          |     |     |            |     |       |
| ADWG<sub>g</sub>, g | 88.92   | 92.09       | 91.20      | 91.29       | 89.67      | 87.23    | 1.233 | 0.072 | 0.120      | 0.855 | 0.063 |
| ADFI<sub>g</sub>, g  | 146.21  | 145.56      | 144.32     | 144.42      | 141.86     | 145.24   | 1.660 | 0.528 | 0.013      | 0.060 | 0.128 |
| FCR<sub>e</sub>   | 1.64<sup>ab</sup> | 1.58<sup>b</sup>| 1.58<sup>b</sup>| 1.58<sup>b</sup>| 1.58<sup>b</sup>| 1.66<sup>a</sup>| 0.021 | 0.013 | 0.009      | 0.060 | 0.128 |
| 0–39 Day     |         |             |            |             |            |          |     |     |            |     |       |
| ADWG<sub>g</sub>, g | 88.92   | 92.09       | 91.20      | 91.29       | 89.67      | 87.23    | 1.233 | 0.072 | 0.120      | 0.855 | 0.063 |
| ADFI<sub>g</sub>, g  | 146.21  | 145.56      | 144.32     | 144.42      | 141.86     | 145.24   | 1.660 | 0.528 | 0.013      | 0.060 | 0.128 |
| FCR<sub>e</sub>   | 1.64<sup>ab</sup> | 1.58<sup>b</sup>| 1.58<sup>b</sup>| 1.58<sup>b</sup>| 1.58<sup>b</sup>| 1.66<sup>a</sup>| 0.021 | 0.013 | 0.009      | 0.060 | 0.128 |
| EEF<sup>g</sup>  | 384.65  | 389.98      | 404.09     | 402.56      | 388.88     | 379.92   | 8.515 | 0.276 | 0.238      | 0.451 | 0.111 |

<sup>a</sup>Values within a row followed by different superscripts are significantly different. <sup>b</sup>p < .05; Tukey’s pairwise test.
<sup>c</sup>Average daily weight gain. <sup>d</sup>Average daily feed intake. <sup>e</sup>Feed conversion ratio. <sup>f</sup>European efficiency factor.
<sup>Trt</sup>: Overall effects of treatments; <sup>C vs. YCWP</sup>: contrasting birds not supplemented with YCWP versus birds supplemented with YCWP; <sup>Lin</sup>: linear effects of increasing inclusion levels of YCWP; <sup>Quad</sup>: quadratic effects of increasing inclusion levels of YCWP.

Results

Growth performance

The effects of adding YCWP to the experimental diets on the performance of the birds during the experimental periods are shown in Table 3. Dietary treatments did not affect ADWG, ADFI, and FCR of the broilers during the starter period (p > .05). The ADWG was similar in different dietary treatments during the grower and finisher periods, but the inclusion of the YCWP linearly decreased ADFI and FCR (p < .05). The same results were found in the overall period, where the lowest FCR was related to the birds fed with 0.45 and 0.6 g/kg YCWP treatments (p < .05). There was no significant difference among experimental diets in terms of EEF during the overall period (p > .05). Contrast analysis also showed that during the grower, finisher and overall periods, adding YCWP to the diets significantly reduced FCR, as compared to the control group (p < .05). Also, with increasing the level of the YCWP in the diet, FCR and ADFI were decreased linearly.

Ileal bacterial population

The effects of dietary inclusion of the YCWP on the microbial population in the ileal content are shown in Table 4. Chickens fed with the 0.45 and 0.6 g/kg YCWP treatments were analysed using the polynomial contrasts. Additionally, the orthogonal contrast was used in SAS 9.4 (2009) to test the control versus YCWP-supplemented birds.

### Table 4. Effects of yeast cell wall product (YCWP) on microbial population in the ileal contents (Log10 CFU/g).

| Treatments | Lactobacillus | Coliform bacteria |
|------------|---------------|-------------------|
| Control    | 7.90<sup>b</sup> | 6.75<sup>b</sup>  |
| 0.15 g YCWP| 8.20<sup>ab</sup>| 6.52<sup>a</sup>  |
| 0.3 g YCWP | 8.03<sup>ab</sup>| 6.46<sup>ab</sup>|
| 0.45 g YCWP| 8.22<sup>b</sup> | 6.23<sup>b</sup>  |
| 0.6 g YCWP | 8.22<sup>b</sup> | 6.45<sup>b</sup>  |
| Colistin   | 8.25<sup>b</sup> | 6.33<sup>ab</sup>|
| SEM        | 0.066         | 0.054             |
| p-Value    |               |                   |
| Trt        | 0.006         | 0.002             |
| C vs. YCWP | 0.035         | 0.003             |
| Lin        | 0.012         | 0.008             |
| Quad       | 0.114         | 0.413             |

<sup>ab</sup>Values within a row followed by different superscripts are significantly different. <sup>b</sup>p < .05; Tukey’s pairwise test.

Statistical analyses

Data were analysed using the GLM procedures of SAS 9.4 statistical software (SAS (Statistical Analysis System) 2009), based on a completely randomised design (CRD). Tukey’s test was then used to determine the difference between treatments. The significance was considered at p < .05. Linear and quadratic effects from feeding control, 0.15 g YCWP, 0.3 g YCWP, 0.45 g YCWP and 0.6 g YCWP treatments were analysed using the polynomial contrasts. Additionally, the orthogonal contrast was used in SAS 9.4 (2009) to test the control versus YCWP-supplemented birds.

Additional statistical analyses were used to compare the performance of the birds during the experimental periods. The difference among experimental diets in terms of FCR was related to the birds fed with 0.45 and 0.6 g/kg YCWP treatments (p < .05). There was no significant difference among experimental diets in terms of EEF during the overall period (p > .05). Contrast analysis also showed that during the grower, finisher and overall periods, adding YCWP to the diets significantly reduced FCR, as compared to the control group (p < .05). Also, with increasing the level of the YCWP in the diet, FCR and ADFI were decreased linearly.

Ileal bacterial population

The effects of dietary inclusion of the YCWP on the microbial population in the ileal content are shown in Table 4. Chickens fed with the 0.45 and 0.6 g/kg YCWP and colistin had higher *Lactobacillus* counts in comparison to those fed the control diet (p < .05). Also,
dietary inclusion of 0.45 g/kg of the YCWP reduced the coliform bacteria counts compared to the control treatment \((p < .05)\). The contrast comparison showed that the addition of YCWPs to the diet significantly increased *Lactobacillus*, while it decreased coliform bacteria than the control birds \((p < .05)\). Also, with increasing the YCWP level in the diet, the population of *Lactobacillus* was increased, while the population of coliform bacteria was decreased linearly \((p < .05)\).

**Immune system responses**

Antibody response data (anti-SRBC) was measured as the serum concentrations of total anti-SRBC antibodies, IgG, and IgM (Table 5). In the primary (29 d) and secondary (36 d) responses, the production of anti-SRBC antibodies was not significantly affected by the dietary treatments \((p > .05)\). However, the results of the secondary response showed that those chickens fed with the YCWP had higher levels of anti-SRBC antibodies and IgM, as compared to the control ones \((p < .05)\). Also, the level of anti-SRBC antibodies and IgM was increased linearly with raising the level of the YCWP in the diet \((p < .05)\). At the end of the experiment, the lymphoid organs were weighted; the related data as a percentage of the live body weight are also presented in Table 5. Chickens fed with 0.45 g YCWP had the highest weight of thymus and bursa of Fabricius \((p < .05)\). Besides, the weight of the thymus was increased linearly with raising the level of the YCWP in the diet \((p < .05)\). The spleen weight was similar in different dietary treatments \((p > .05)\).

**The concentration of cecal SCFAs**

The concentration of SCFAs (acetate, propionate, butyrate, isobutyrate, valeric, isovaleric and total volatile fatty acids) in the caecum on the 40th day can be

Table 5. Effect of yeast cell wall product (YCWP) and antibiotic in response to SRBC and weight of lymphoid organs at the end of the experiment.

| Treatments | Primary response (29 d) | Secondary response (36 d) | Weight of lymphoid organs (% of live BW) |
|------------|------------------------|---------------------------|----------------------------------------|
|            | Total IgG | IgM | Total IgG | IgM | Spleen | Bursa of Fabricius | Thymus |
| Control    | 3.2      | 1.9 | 1.3     | 4.2 | 2.7    | 1.6              | 0.091 | 0.143 b | 0.345 b |
| 0.15 g YCWP| 4.0      | 2.2 | 1.8     | 5.1 | 3.2    | 1.9              | 0.103 | 0.159 ab| 0.381 ab |
| 0.3 g YCWP | 4.0      | 2.1 | 1.9     | 5.9 | 3.3    | 2.6              | 0.101 | 0.142 b| 0.325 b |
| 0.45 g YCWP| 4.2      | 2.4 | 1.8     | 5.9 | 3.1    | 2.8              | 0.106 | 0.194 a| 0.434 a |
| 0.6 g YCWP | 4.4      | 2.6 | 1.9     | 5.8 | 3.2    | 2.6              | 0.098 | 0.151 ab| 0.370 ab |
| Colistin   | 3.7      | 2.7 | 1.9     | 4.9 | 3.6    | 2.8              | 0.106 | 0.159 ab| 0.355 ab |
| SEM        | 0.64     | 0.43| 0.35    | 0.58| 0.42   | 0.34             | 0.0058| 0.0109  | 0.0157 |

Values within a column followed by different superscripts are significantly different. \(p < .05\); Tukey’s pairwise test.

Trt: overall effects of treatments; C vs. YCWP: contrasting birds not supplemented with YCWP versus birds supplemented with YCWP; Lin: linear effects of increasing inclusion levels of YCWP; Quad: quadratic effects of increasing inclusion levels of YCWP.

Table 6. Effect of adding yeast cell wall product (YCWP) to the diets on the concentration of short-chain fatty acid in the caecum at 40 days of age (mmol/g).

| Treatments | Acetate | Propionate | A:P ratio* | Butyrate | Valerate | Isovalerate | Total FA |
|------------|---------|------------|------------|----------|----------|-------------|----------|
| Control    | 42.31   | 4.64       | 10.02      | 7.12     | 0.206    | 0.492       | 0.543    | 55.32 |
| 0.15 g YCWP| 43.85   | 5.88       | 8.03       | 5.08     | 0.244    | 0.538       | 0.566    | 56.16 |
| 0.3 g YCWP | 45.41   | 6.06       | 8.11       | 5.00     | 0.249    | 0.538       | 0.586    | 57.68 |
| 0.45 g YCWP| 47.33   | 7.02       | 7.24       | 5.27     | 0.341    | 0.669       | 0.688    | 61.33 |
| 0.6 g YCWP | 48.41   | 7.14       | 7.18       | 6.13     | 0.343    | 0.680       | 0.689    | 63.40 |
| Colistin   | 49.37   | 6.14       | 8.58       | 6.33     | 0.235    | 0.516       | 0.574    | 63.38 |
| SEM        | 4.88    | 0.731      | 1.047      | 1.224    | 0.067    | 0.146       | 0.100    | 6.104 |

\*Acetate to propionate ratio.

Trt: Overall effects of treatments; C vs. YCWP: contrasting birds not supplemented with YCWP versus birds supplemented with YCWP; Lin: linear effects of increasing inclusion levels of YCWP; Quad: quadratic effects of increasing inclusion levels of YCWP.
seen in Table 6. The SCFAs concentration was not affected by the dietary treatments \((p > .05)\). However, the addition of YCWP increased the propionate concentration, as compared to the control group \((p < .05)\); consequently, it tended to decrease the acetate to propionate ratio \((p = .055)\). Also, the concentration of propionate in the caecum was increased linearly with raising the level of the YCWP in the diet \((p < .05)\).

### Histological changes of the jejunum

The results of the assessed histological parameters of the jejunum (villus height, crypt depth, villus width, villus surface area, and muscle thickness) are shown in Table 7 and Figure 1. Adding the YCWP to the diet increased the villus height linearly \((p < .05)\). The highest and shortest villus height was observed in the birds fed with 0.6 g of the YCWP and colistin, respectively \((p < .05)\). The control birds had the deepest crypt, as compared to those fed the diets containing colistin and the YCWP \((p < .05)\). Also, the highest and lowest VH: CD ratio was related to 0.6 g YCWP and control treatments, respectively \((p < .05)\). Furthermore, with increasing the level of the YCWP in the diet, the width of the intestinal villus was increased linearly \((p < .05)\); so, the maximum villus width was observed in the birds receiving 0.45 and 0.6 g of the YCWP, while the lowest villus width was observed in those fed the control, 0.15 and 0.3 g YCWP. Chickens fed with 0.6 g YCWP had the highest villus surface area, while those using colistin and 0.15 g of YCWP had the lowest villus surface area \((p < .05)\). The muscle thickness was similar in treatments \((p > .05)\). According to the results of the contrast analysis, birds whose diet was supplemented with YCWP had a lower crypt depth and higher VH: CD, as compared to those receiving the control diet \((p < .05)\). Also, with the increasing the YCWP level in the diet, VH: CD and villus surface area were increased linearly, while the crypt depth was decreased linearly and quadratically \((p < .05)\).

### Gene expression

The results of the gene expression are presented in Figure 2. Feeding birds with 0.15 and 0.3 g of the YCWP increased \(PPAR\_\gamma\) transcription, while the inclusion of the YCWP at the level of 0.45 and 0.6 g did not change the \(PPAR\_\gamma\) gene expression, as compared to the control birds \((p < .05)\). As demonstrated in Figure 2(B), the \(PGC1-\alpha\) mRNA level was increased in the chickens fed with 0.15 g YCWP \((p < .05)\). However, birds fed the diet containing other levels of the YCWP did not change \(PGC1-\alpha\) mRNA expression, as compared to the others \((p < .05)\). Colistin did not change \(PGC1-\alpha\) gene expression than the control group. The \(TLR4\) gene expression in 0.3 and 0.45 g YCWP-fed birds was significantly lower than that in the control birds \((p < .05)\). However, the inclusion levels of 0.15 and 0.6 g YCWP did not change the gene expression of \(TLR4\), as compared to the control birds \((p < .05)\). The effects of the dietary treatments on the gene expression of \(IL-10\) are presented in Figure 2(D). As expected, feeding birds with 0.15 and 0.3 g of YCWP/kg of the diet resulted in the greater transcription of the \(IL-10\) gene. However, at the greater inclusion levels \((0.45 \text{ and } 0.6 \text{ g/kg of diet})\), YCWP supplementation and colistin did not change the \(IL-10\) expression, as compared to the control group \((p < .05)\). The orthogonal contrasts showed that the mRNA for \(PPAR\_\gamma\) \((p = .021)\), \(PGC1-\alpha\) \((p = .000)\) and \(IL-10\) \((p = .012)\) were elevated in the YCWP group, while \(TLR4\) mRNA \((p = .002)\) was decreased with adding YCWP compared to the control diet (Figure 3).
Discussion

In this study, the effects of the dietary inclusion of YCWP on the growth performance of the broiler chickens reared under normal physiological conditions were evaluated. Prebiotics and YCWP supplementation in the broiler chickens generally improves their performance (Teng and Kim 2018; Ricke et al. 2020); however, in this experiment, YCWP (MOS and β-Glucan) supplementation only improved the FCR of chickens. These results were in agreement with those of Pourabedin et al., reporting that adding MOS to the broilers’ diet had no significant effect on their daily weight gain. However, in their study, YCWP supplementation did not influence the FCR of 35-d-old broilers, which was in contrast with our findings (Pourabedin et al. 2014). Similar results have also been obtained in the birds fed with MOS and grown under an experimentally controlled condition (Sims et al. 2004). Improving the performance of broiler chickens by dietary supplementation with β-glucans or MOS could be mainly attributed to the modulation of the intestinal microbiota and enhanced innate immune function (Kim et al. 2011). For example, in a recent study, it was reported that adding prebiotics (containing 2.6 Beta LevaFructan 100 gm on 1000 mL distilled water) to the water reduced the adverse effects of Salmonella and increased the performance of broiler chickens during the bacterial challenge (El-Shall et al. 2019). It has also been reported that prebiotics (isomalto-oligosaccharide) could significantly improve the growth performance and modulate the broiler chickens’ intestinal microbiota when challenged by E. coli O78 (Tarabees et al. 2019). In the present study, the experimental birds were reared in a controlled environment and not exposed to any exogenous bacteria or environmental challenge; this may explain why adding the YCWP to the diets had no effect on the bird’s performance in the present study (Wang et al. 2016).

In general, yeast cell wall products and prebiotics modulate the microbial population of GIT by increasing the number of beneficial bacteria such as Lactobacillus and Bifidobacteria and decreasing such pathogenic bacteria as Salmonella and E. coli (Zoumpopoulou et al. 2018). In this experiment, we observed that with increasing the YCWP levels in the diet, the number of Lactobacillus in the ileum was significantly increased, resulting in decreasing the number of coliform bacteria in the ileal content (Table 4). Prebiotics could affect the gut microbiota in several ways: 1. providing the nutrients for the growth of

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**Figure 1.** Effect of yeast cell wall product (YCWP) and antibiotic on jejunal histological changes in broilers at 40 d of age. Arrows: (a) villus height; (b) villus width; (c) crypt depth; d: muscle thickness.
commensal bacteria, 2. inhibiting the growth of pathogenic bacteria by increasing the development of commensal bacteria and creating competitive exclusion between them, and preventing the attachment of pathogenic bacteria to the intestinal epithelial sites (Pourabedin and Zhao 2015; Adhikari and Kim 2017), and 3. binding to the pathogenic bacteria, which possess type-1 fimbriae such as E. coli and Salmonella species. Mannan oligosaccharides have receptor properties for E. coli and Salmonella’s fimbriae that can prevent them from attaching to the intestinal epithelium and removing with the digesta flow; MOS can also block the bacterial lectins and reduce the colonisation of these pathogens in the bird’s GIT (Fernandez et al. 2002; Teng and Kim 2018).

In this experiment, YCWP had no significant effect on the antibody production against SRBC, as compared to the control group (Table 5). However, the heavier bursa of Fabricius and thymus weight in YCWP-fed birds indicated the improved immune system function. This might be due to a direct interaction between prebiotics and host GIT immune cells, as well as the indirect effects of prebiotics on the immune system by modulating the intestinal microbiota and increasing the beneficial bacteria (Janardhana et al. 2009). Microbiota such as Lactobacillus and Bifidobacterium support the animal’s immune system against invasive pathogens by stimulating the GIT immune responses. Prebiotics indirectly strengthen the bird’s immune system by stimulating the growth of these bacteria. Also, some YCWPs such as MOS, can interact directly with antigens and increase the immune signals in chickens (Teng and Kim 2018). Generally, prebiotics can strengthen the host immune system in several ways. Firstly, this can be through their effects on the lymphoid tissues of chickens, such

Figure 2. The expression of genes that are involved in inflammation and metabolism in ileum tissue of broiler chickens. (A) PPARγ gene expression. (B) PGC-1α gene expression. (C) TLR4 gene expression. (D) IL-10 gene expression.
as the caecal tonsil, spleen, thymus, and bursa of Fabricius (Table 5). Secondly, this can be realised by enhancing the antibody titres of IgM and IgG in plasma and IgA level in the intestine. Thirdly, it could be due to the enhanced phagocytosis and proliferation of monocytes and macrophages. As the fourth way, increasing the expression of mucin genes in the gut can be noted. Finally, the GIT immune functions could be enhanced (Huang et al. 2015; Adhikari and Kim 2017). In the present study, the lymphatic organs were weighed at the end of the rearing period. The weight of the lymphatic organs can indicate the general condition and health of the birds. In chickens, the bursa of Fabricius and thymus can be regarded as the sites for T and B lymphocytes maturation; therefore, the size and weight of the bursa and thymus can provide important information about the growth and development of the immune system (Grasman 2002). Generally, when the bursa of Fabricius weight to body weight ratio is about 0.2%, it can be concluded that the bird has an immune system with a good performance and health (Sellaoui et al. 2012). In our study, the increase in the thymus and bursa weight could indicate the development of the bird’s immune system due to the use of YCWP.

One of the positive effects of YCWP is increasing the production of short-chain fatty acids (specifically propionate, acetate and butyrate) in the caecum by stimulating the growth of gastrointestinal bacteria. However, in the present study, YCWP supplementation had no significant effect on the caecal concentration of SCFAs. According to Table 6, the YCWP could numerically increase the concentration of propionate in the caecum. In addition to the reasons mentioned earlier, the reduction of pathogenic bacteria in the intestine could be attributed to the decrease of pH due to the increased SCFAs in the intestine (Micciche et al. 2018; Kim et al. 2019).

This study showed that adding the YCWP to the diet increased the villus height and reduced the crypt depth (Table 7). The effect of YCWP on intestinal morphology is probably due to its impact on the GIT microbiota. Some YCWPs, such as MOS, can improve the villi growth and structure in the small intestine and increase the activity of such jejunal enzymes as maltase, aminopeptidase, and alkaline phosphatase (Ricke et al. 2020). It has been shown that adding MOS to the broilers’ diets could increase Lactobacillus in the ileum and, consequently, the activity of digestive enzymes in the chickens. Clavijo and Flórez have also reported that the activity of digestive enzymes is affected by the intestinal microflora. For example, Bifidobacterium and Lactobacillus increase the activity of digestive enzymes such as proteases and lipases (Clavijo and Flórez 2018). Also, the higher levels of intestinal Lactobacillus in the birds supplemented with MOS resulted in a healthier GIT (Teng and Kim 2018). The intestinal microbiota balance and intestinal morphology, including the villus height, crypt depth and villus height to crypt depth ratio, are the critical indices of gut health and function. Previous studies have also reported that MOS supplementation increases the villus height and surface area, decreases the crypt depth, and up-regulates the gene expression of mucin genes related to mucin secretion (Yang et al. 2008; Baurhoo et al. 2009; Chee et al. 2010). Improved intestinal morphology in MOS-fed chickens may also be due to reducing the population of pathogenic bacteria in the GIT. For instance, some pathogenic bacteria, such as E.coli, can impair the digestive enzymes’ secretion in two ways. Firstly, this is done by secreting the microbial proteolytic enzymes that degrade the host digestive enzymes; secondly, this occurs by damaging the intestinal villus and microvillus (Pan and Yu 2014).

Previous reports have also shown that inflammation induces the production of reactive oxygen species
(ROS) in the mitochondria and, finally, in the cells. However, almost all cells produce modulators through the expression of genes, which regulate the production of inflammatory factors such as ROS. The uncoupling proteins (UCP) are mitochondrial transporters present in the inner membrane of mitochondria (Ledesma et al. 2002). Some subsets of UCP (UCP-2 and UCP-3) protect the cells against the oxidative damage of superoxide by the mild uncoupling (Brand et al. 2002, 2004). PGC-1α is the most dominant regulatory protein in mitochondrial biogenesis (Wu et al. 1999). PGC-1α stimulates the nuclear respiratory factor-1 and the mitochondrial transcription factor expression; these, in turn, can lead to up-regulating the expression of nuclear and mitochondrial genes that encode the mitochondrial proteins (Andersson and Scarpulla 2001; Nisoli et al. 2003). As illustrated in Figure 2(B), the dietary supplementation of broiler chickens with the probiotic at 0.15 g/kg level resulted in the greatest PGC1-α transcription among birds. It has also been reported that the increased UCP could significantly down-regulate the ROS production following fasting (Abe et al. 2006). Also, acute heat stress induces mitochondrial superoxide production in the broiler skeletal muscle through the down-regulation of UCP (Mujahid et al. 2006). Besides, some previous studies have suggested that PPARγ, PGC-1α, and nitric oxide play an important role in mitochondrial biogenesis (Wu et al. 1999; Nisoli et al. 2003). Nisoli et al. (2003) reported that NOS-/- mice had lower PGC-1α mRNA levels than the wild-type ones. It has also been shown that the exposure of chickens to a cold environment results in the prompt up-regulation of the PGC-1α expression (Ueda et al. 2005). Therefore, the up-regulation of PGC-1α may up-regulate such PGC-1α dependent factors as UCPs; these may, in turn, decrease oxidative stress and, at the next level, increase mitochondrial biogenesis.

The family of peroxisome proliferation-activated receptors includes three isoforms: PPARα, PPARβ, and PPARγ (Berger and Moller 2002). The PPARγ is expressed in white and brown adipose tissues and the large intestine. In the molecular epithet, PPARs are a family of ligand-activated nuclear hormone receptors (NRs) belonging to the steroid receptor superfamily. PPARs are nuclear receptor proteins involved in many regulatory functions (Desvergne and Wahli 1999). PPARγ is the dominant regulator of the expression of genes encoding proteins that control adipocyte differentiation and fatty acids uptake and metabolism (Kliwer et al. 1997; Rosen et al. 1999). In chickens, PPAR-γ plays a crucial role in fat deposition (Sato et al. 2004). It was reported that dietary supplementation of sphingomyelin reduces inflammation in the intestinal epithelial cells through the upregulation of PPARγ (Mazzei et al. 2011). Many studies reported that PPARγ regulates a large number of activities such as glucose and lipid metabolism, cellular proliferation, differentiation, and inflammation (Auwerx 2002; Dubuquoy et al. 2006; Leonardini et al. 2009; Duszka et al. 2016). The PPARγ is highly expressed in the GIT; PPARγ is expressed in the duodenum and especially in the proximal colon (Mansen et al. 1996; Escher et al. 2001; Harmon et al. 2010). The PPARγ transcription and activity are stimulated in the GIT by numerous bacterial metabolites and bacterial by-products such as butyrate (Wachtershauser et al. 2000; Schwab et al. 2007), H2O2 (Voltan et al. 2008), and LPS (Dubuquoy et al. 2003). Expression of PGC-1α mRNA was not different among control, antibiotic group, and birds supplemented with high levels of the YCWP in the ileal tissue (Figure 2(B)). However, we observed a higher level of PPARγ mRNA in the birds fed the diet containing 0.15 and 0.3 g/kg YCWP, thus implying that a threshold of PPARγ transcription and finally PPARγ protein expression would be needed for triggering the functional roles of PGC-1α. Also, as illustrated in Figure 2(A,B), in the birds fed with 0.45 and 0.6 g/kg YCWP, PPARγ and PGC-1α mRNA were the lowest; this might indicate the reduced inflammation in their intestinal cells. Besides, the greater PGC-1α mRNA in 0.15 g/kg YCWP -fed birds reflected the improved metabolism and biogenesis in their intestinal cells. On the other hand, one of the critical roles of PPARγ is to prevent lipotoxicity by different mechanisms and to regulate the targets relevant to inflammation (Clark 2002). When a YCWP with a specific function destroys the pathogenic bacteria and their pieces, the endothelial cells prefer to increase the homeostasis instead of activating the anti-inflammation cascade. A previous study showed that PPARγ agonists could increase anti-inflammatory factors such as adiponectin and reduce tissue necrosis factors (Krishnaswami et al. 2010). Researchers have investigated the effects of PPARγ activation on the intramuscular fat in the growing beef bulls, using the oral administration of 2, 4-thiazolidinedione (TZD) to activate PPARγ. They have shown that the oral administration of TZD did not affect the carcase quality, but it could decrease the expression of PPAR (α, -δ and, -γ) in the liver (Arevalo-Turrubiarte et al. 2012). Our results also demonstrated that feeding birds with the YCWP at the levels of 0.45 and 0.6 g/kg decreased the inflammation, indicating that the IL-10 and PPARγ expression were similar to control
in their ileum; finally, improving metabolism in them (Janani and Kumari 2015). However, as displayed in Table 7 and Figure 1, with increasing the YCWP in the broilers diet, the histological changes of the jejunum were considerably better than those in the control and antibiotic groups. However, we demonstrated that in the lowest level of the YCWP, the PPARγ and PGC-1α expression was significantly increased. These all indicate that PPARγ and PGC-1α may be involved in the proliferation of the GIT and goblet cells in the crypt depth. Some researchers have also shown that PGC-1α is one of the critical factors in cell proliferation and differentiation (Zheng et al. 2016).

Toll-like receptors, also known as pathogen recognition receptors, are part of the innate immune system (Aderem and Ulevitch 2000). These receptors recognise the molecular patterns of pathogens called microbe-associated molecular patterns, initiate a chain reaction, and stimulate the immune system (Aderem and Ulevitch 2000). Chicken TLR2b4, and 21 are involved in recognising peptidoglycan, lipopolysaccharides, and unmethylated CpG oligonucleotides, respectively (Hoshino et al. 1999; Fukui et al. 2001; Keestra et al. 2010). Yitbarek et al. (2013) showed the greater expression of TLR4, and TLR2b in the bird’s spleen and bursa of Fabricius fed with yeast culture-supplementation diets (Yitbarek et al. 2013). They also demonstrated that the dietary supplementation with a yeast culture in chickens significantly increased the TLR21 transcription in their intestine.

However, in the current study, the lower concentration of the YCWP did not restrain the harmful bacteria (Coliform bacteria). But the higher inclusion levels (0.3, 0.45, and 0.6 g/kg of YCWP) resulted in the lower harmful bacteria and finally led to a reduction of TLR4 transcription and inflammation. The linkage between TLR4 and LPS could activate the cascade of the signaling pathway in the inflammation, subsequently increasing the anti-inflammatory cytokine expressions, such as IL-10, which are involved in the immune response and the intrinsic immune system (Chen et al. 2010). The IL-10 is an anti-inflammatory cytokine that acts as a potential factor in decreasing the inflammation and host pathology when diseases occur (Redpath et al. 2014). Dependent protozoan parasites activate IL-10 to diminish the pathogen-damaging host inflammatory responses. Arendt et al. also showed that the Eimeria challenge in broiler chickens increased the IL-10 gene expression (Arendt et al. 2019). Some studies have demonstrated that IL-10 acts as a macrophage-inactivating regulatory cytokine, suppressing the secretion of the pro-inflammatory cytokine and inhibiting the expression of the major histocompatibility class II (MHC II) and co-stimulatory molecules (O’Garra and Vieira 2007). In healthy chickens, the gene expression of IL-10 mRNA has been shown to be greater in the duodenum than in the caecum (Haritova and Stanilova 2012). Yitbarek et al. (2013) also observed the up-regulation of the IL-10 gene only in the ileum of the birds receiving a yeast culture supplemented diet, which can be considered as a local inhibition of the Th1 pathway (Yitbarek et al. 2013). He et al. (2012) have also suggested that the regulation of the macrophage activity by Th1/2 cytokines is crucial to maintain a balanced immunity, providing adequate Th1-mediated protective immunity and preventing the detrimental effects of severe inflammation (He et al. 2012). Therefore, these results indicated that dietary YCWP supplementation at the level of 0.6 g/kg considerably reduced the chronic inflammation in the broilers’ GIT; meanwhile, this led to the successful regulation of the metabolism and specifically, mitochondrial biogenesis. In other words, the inclusion of the YCWP in the broiler diets could modulate the local innate immunity in the broiler chickens. Besides, feeding YCWP-supplemented diets for a long period might have decreased the bacterial population, TLR4 responses, and inflammation.

Conclusions
The results demonstrated that dietary YCWP supplementation could positively affect the broilers’ gut, epithelial cell differentiation and turnover, and immunomodulation. Furthermore, the YCWP improved intestinal villi development, which could be due to the PPARγ gene expression and the increasing population of the beneficial bacteria, specifically Lactobacillus. Supplementation of YCWP regulated pro and anti-inflammatory cytokines via increment of IL-10 and reduction of TLR4 expression systemic pathways. Therefore, YCWP supplementation could have an immune stimulatory effect via the local and systemic responses involving TLRs and cytokines, with implications for enhancing the resistance to the pathogenic microorganisms. Furthermore, because the jejunum is a key organ in controlling the entry of bacteria and other pathogens entering into the GIT, the strong responses of the cytokines observed in the current study warrant further investigation into the role of YCWP in limiting the pathogens’ invasion.

Ethical approval
The authors confirm that THEY were followed the animal welfare guide, as adopted by FASS (2010). All animal care
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Disclosure statement

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