Effects of a combination of phytase and multi-carbohydrase enzymes in low-density corn–soybean meal based diets on growth performance and ileal nutrients digestibility of male broilers

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ABSTRACT
A study was conducted to evaluate the effect of dietary supplementation of phytase and carbohydralase on growth performance and ileal digestibility of nutrients in broilers fed low-density corn–soybean meal-based diets. A total of 280 one-day-old Ross 308 male broilers were randomly assigned to 7 dietary treatments, with 4 replicates of 10 birds. Dietary treatments were (1) full nutrient specification without enzyme supplementation (positive control; PC), (2) diet with reduced nutrients as a phytase matrix (negative control; NC), (3) NC + phytase (500 FTU/kg diet), 4) NC + multi-carbohydrase (0.1 g/kg), (5) NC + phytase + multi-carbohydrase, (6) diet with reduced nutrients as phytase and multi-carbohydrase matrices; low negative control (LNC), and (7) LNC + phytase + multi-carbohydrase. Broilers fed either NC or LNC diets had lower body weight gain, the European production efficiency factor, and a higher feed conversion ratio than those fed PC diet (p < .01). Broilers fed LNC diet had lower dry matter (DM) and crude protein (CP) digestibility, and apparent ileal digestible energy (AIDE) than those fed PC diet (p < .01). Multi-carbohydrase supplementation on top of phytase-containing NC diets resulted in a similar effect on performance and digestibility of DM and AIDE in broilers compared with LNC diets containing phytase and multi-carbohydrase. Digestibility of CP was lower in NC + multi-carbohydrase groups than NC + phytase + multi-carbohydrase (p < .01). In conclusion, multi-carbohydrase was successful in improving broiler performance and nutrients digestibility in the diet based on the corn–soybean meal with lower nutrient density; thus, when supplementing it as a second enzyme in diets containing phytase, its matrix value should be considered for least-cost feed formulation.

HIGHLIGHTS
- Phytase and carbohydrate matrix values should be considered when enzyme combination using in C-SBM diet.
- Carbohydrase improved broiler performance and nutrients digestibility in C-SBM diet with lower nutrient density.
- Cost-effectiveness use of enzyme combination in diets could reduce nutrient excretion.

Introduction
The feed is the major cost item in poultry production, with the most economically interesting dietary components being energy, amino acids, and phosphorus, respectively. Feed enzymes application is an effective tool to increase nutrient digestibility, thereby decreasing nutrient excretion as well as reducing feed cost (Bedford and Partridge 2010). The most extensively used exogenous enzyme in the poultry industry is phytase (Attia et al. 2019). The positive effects of using phytase in poultry feed on reducing the antinutritional effects of phytate and improving the digestibility of phosphorous, minerals, amino acids and energy are well recognised (Selle and Ravindran 2007). In poultry diets, particularly those based on wheat and barley, the use of carbohydrases (e.g. β-glucanase and xylanase) has also been shown to have benefits, including hydrolysing non-starch polysaccharides.
(NSP), decreasing digesta viscosity and releasing entrapped nutrients and improving nutrient utilisation and growth performance (Masey-O’Neill, Smith, et al. 2014; Attia et al. 2020). However, in low viscosity corn–soybean meal-based diets (C-SBM diets), also carbohydrases may be desired to ensure breakdown of the cell wall matrix, and release nutrients encapsulated in the cell wall of the corn and SBM (Slominski 2011). Oligosaccharides, especially α-galactosides, are more prevalent originated from SBM (Knudsen 2014). Dietary α-galactosidase supplementation potentially improves nutrient utilisation and absorption, which could lead to better performance (Pettersson and Pontoppidan 2013).

Reviewing the literature indicates that most published research studies have individually investigated carbohydrases, while commercial diets typically contain phytase concurrently with carbohydrates. It is hypothesised that phytase and carbohydrases can act synergistically in improving nutrient utilisation (Woyengo et al. 2010; Attia et al. 2012). Essentially, the carbohydrases can hydrolyse NSP in plant cell walls to increase phytase access to phytate within the cells (Woyengo and Nyachoti 2011). Enzymes are not typically added on top of diets because of cost considerations. In commercial practice, the use of enzymes is often accompanied by using a nutrient matrix value, to ensure both economic and nutrition considerations. In the flip side, cost savings may be reduced, or even lost, if matrix values are incorrectly chosen (Adeola and Cowieson 2011). Currently, there are limited applied research findings to be used by the poultry industry when a combination of these two enzymes is being considered in feed formulation. Phytase and carbohydrases are often used in a commercial diet, each of which has a matrix value that should ensure their matrices are additive before being incorporated in the feed formulation. Therefore, the objective of the current study was to investigate the interaction of phytase and multi-carbohydrase in low-density C-SBM diets on growth performance and ileal digestibility of nutrients in broilers.

Materials and methods

Birds management and housing

The management of birds and all experimental procedures performed in the study were approved by the Animal Care and Use Committee of the University of Guilan, Iran. A total of 280 one-day-old Ross 308 male broiler chicks, with an average body weight of 41.3 ± 0.7 g were randomly distributed into 28-floor pens (1 m × 1 m). Chicks were placed on clean soft-wood shavings and each pen was equipped with one hanging around the feeder and one bell drinker. Room temperature was kept at 32 °C during the first 7 d of age, and then gradually decreased, reaching a final temperature of 23 °C at 21 d of age. There was a continuous light regimen during the first 2 d, then 23 h of lighting up to the age of 42 d was applied. Light intensity was 20 lux at the first 2 d then stepped down to 5 lux for the remaining period (3–42 d).

Diets and experimental design

The experiment was performed as a completely randomised design with 7 treatments and 4 replicates of 10 chicks per replication. Dietary treatments included: (1) Positive control (PC; a nutritionally adequate diet according to Ross 308 recommendations; Ross Broilers Ltd 2014), (2) Negative control (NC; diet with reduced nutrients as the equivalency recommended by phytase matrix value: P 0.115%; Ca 0.1%; Digestible Lys 0.012%; Digestible Met + Cys 0.0044%; Digestible Thr 0.013%; CP 0.225%; ME 53 kcal/kg), (3) NC + phytase (500 phytase units; FTU/kg diet; Natuphos TM; 10,000 BASF Corp., Germany), (4) NC + multi-carbohydrase (0.1 g/kg; Endo-power TM; Easy Bio Inc., South Korea), The enzyme complex of multi-carbohydrase contained 35 unit/g α-galactosidase activity, 110 unit/g galactomannanase activity, 1500 unit/g xylanase activity and 1100 unit/g β-glucanase activity, (5) NC + phytase + multi-carbohydrase, (6) Low negative control (LNC; diet with reduced nutrients content according to multi-carbohydrase matrix value provided by Easy Bio Inc, South Korea: Digestible Lys 0.088%; CP 0.2%; ME 50 kcal/kg and phytase matrix value) and (7) LNC + phytase + multi-carbohydrase.

The ingredients and nutrients content of the basal diets were presented in Table 1. Three-phase feeding programs, including starter (1–10 d), grower (11–24 d) and finisher (25–42 d) were provided in mash form. Feed and freshwater were offered ad libitum throughout the experiment. All grower diets contained 2%
Table 1. Composition and calculated nutrient composition of the basal diets\(^a\) (as fed basis, %).

| Item                  | PC     | NC     | LNC    |
|-----------------------|--------|--------|--------|
| **Starters (1–10 d)** |        |        |        |
| Corn                  | 53.31  | 55.95  | 57.19  |
| Soybean meal, 44% CP  | 39.23  | 38.49  | 38.27  |
| Soybean oil           | 2.91   | 1.45   | 0.44   |
| Dicalcium phosphate   | 2.03   | 1.66   | 1.66   |
| Calcium carbonate     | 0.98   | 0.92   | 0.92   |
| Common salt           | 0.24   | 0.24   | 0.24   |
| L-Lysine HCI, 78%     | 0.23   | 0.23   | 0.22   |
| DL-Methionine, 98%    | 0.32   | 0.31   | 0.31   |
| L-Threonine, 99%      | 0.10   | 0.10   | 0.10   |
| Vitamin premixb       | 0.25   | 0.25   | 0.25   |
| Mineral premixc       | 0.25   | 0.25   | 0.25   |
| Sodium bicarbonate    | 0.15   | 0.15   | 0.15   |
| Celite                | 0.00   | 0.00   | 0.00   |
| **Total**             | 100    | 100    | 100    |
| **Nutrient composition (calculated)** |        |        |        |
| Metabolisable energy, kcal kg\(^{-1}\) | 2900 | 2850 | 2800 |
| Crude fibre, %        | 5.48   | 4.11   | 3.14   |
| Ether extract, %      | 3.64   | 3.66   | 3.67   |
| Crude protein, %      | 22.2   | 22.1   | 22.1   |
| Dig. lysine, %        | 1.24   | 1.23   | 1.22   |
| Dig. methionine, %    | 0.62   | 0.61   | 0.61   |
| Dig. methionine + cystine, % | 0.92 | 0.91 | 0.91 |
| Dig. threonine, %     | 0.83   | 0.82   | 0.82   |
| Calcium, %            | 0.93   | 0.83   | 0.83   |
| Available phosphorus, % | 0.48 | 0.41 | 0.41 |
| Total phosphorus, %   | 0.75   | 0.69   | 0.69   |
| Sodium, %             | 0.15   | 0.15   | 0.15   |
| Potassium, %          | 0.91   | 0.90   | 0.90   |
| Chloride, %           | 0.23   | 0.23   | 0.23   |
| **Nutrient composition (analysed)** | 88.54 | 88.65 | 88.49 |
| Dry matter, %         | 19.6   | 21.7   | 21.6   |
| Gross energy, kcal kg\(^{-1}\) | 4292 | 4216 | 4153 |
| Crude protein (nitrogen × 6.25), % | 24.66 | 24.65 | 24.65 |

*PC: positive control (a nutritionally adequate diet); NC: negative control (deficient in nutrients level according to phytase matrix provided by BASF, Germany); LNC: low negative control (deficient in nutrients level according to phytase and multi-non-starch polysaccharides enzyme matrix provided by BASF, Germany and Easy Bio Inc., South Korea). Vitamin premix provided the following per kilogram of diet: vitamin A (trans-retinyl acetate), 10,000 U; vitamin D\(_3\) (cholecalciferol), 2000 U; vitamin E (DL-\(\alpha\)-tocopherol acetate), 10,000 U; vitamin K\(_3\) (bisulfate menadione complex), 5 mg; thiamine (thiamine mononitrate), 3 mg; riboflavin, 9 mg; nicotinic acid, 30 mg; pantothenic acid (D-calcium pantothenate), 10 mg; vitamin B\(_6\), 4 mg; D-biotin, 0.1 mg; folic acid, 2 mg; vitamin B\(_12\) (cyanocobalamin), 0.02 mg and choline (choline chloride), 1000 mg. *Mineral premix provided the following per kilogram of diet: iron (FeSO\(_4\)•7H\(_2\)O), 55 mg; iodine (Ca (IO\(_3\))\(_2\)), 1.3 mg; manganese (MnSO\(_4\)•H\(_2\)O), 100 mg; zinc (ZnO), 85 mg; copper (CuSO\(_4\)•H\(_2\)O), 13 mg; selenium (Na\(_2\)SeO\(_3\)), 0.2 mg. *The analysed values represent the mean of duplicate samples per analysis.

Celite (Celite Hispánica S.A., Alicante, Spain) as an indigestible marker to measure nutrient digestibility.

**Collection of samples and measurements**

Bodyweight of the birds and feed intake were recorded by pen, and mortality was recorded and weighed as it occurred. Bodyweight gain (BWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated during the experimental periods. At the end of the experiment (day 42), the European production efficiency factor (EPEF) was calculated using the following formula: BW (kg) × % liveability × 100/FCR × trial duration (d) (Huff et al. 2013). At 21 d of age, two birds per replicate were killed by cervical dislocation and digesta from the distal half of ileum (from the middle of the ileum to approximately 1 cm above the ileocecal junction) was collected, pooled and stored in airtight containers at −20°C until laboratory analysis.

**Laboratory analysis and calculations**

Dry matter (DM) content of the diets and ileal samples were determined by drying the samples in a drying oven at 105°C for 24 h using a standard procedure (Methods 930.15; Association of Official Analytical Chemists 2005). Total nitrogen (N) content of the diets and ileal samples was determined following the Kjeldahl procedure with digestion on a Gerhardt Vapodest 45 device (Gerhardt GmbH and Co. KG, Germany) and crude protein (CP) was calculated as N × 6.25. Ileal digesta and samples of the diets were analysed for gross energy (GE) value using an adiabatic bomb calorimeter calibrated with benzoic acid as an internal standard (IKA Calorimeter System, IKA-Werke C 5003/C 5001, GmbH & Co. KG, Staufen,
Acid insoluble ash concentration in the diets and ileal samples were determined using the method described by De Coca-Sinova et al. (2011). The apparent digestibility values of DM and CP were calculated as follows (Stefanello et al. 2015):

\[
\text{Digestibility (\%) } = \left(1 - \left[\frac{\text{Md} \times \text{Ni}}{(\text{Mi} \times \text{Nd})}\right]\right) \times 100
\]  

Where Md is the concentration of an indigestible marker (acid insoluble ash) in diet; Ni is the DM, CP and GE concentration in ileal digesta; Mi is the acid insoluble ash concentration in ileal digesta; and Nd is the DM, CP and GE concentration in diet. Apparent ileal digestible energy (AIDE) was calculated by multiplying the diet GE content by the apparent ileal energy digestibility coefficient (Amerah et al. 2017).

### Results

#### Performance

From 1 to 21 d of age, broilers fed either NC or LNC diets had lower \((p < .01)\) BWG and higher FCR \((p < .01)\) than those fed PC diets (Table 2). There was no significant difference in BWG and FCR between the birds fed NC + phytase diet and those fed PC diets. No increase in BWG and FCR was observed by multi-carbohydrase supplementation without reducing its matrix value in phytase containing diet (NC + phytase + multi-carbohydrase), compared with those fed NC + phytase diet. However, when the multi-carbohydrase matrix value was reduced (LNC), birds fed multi-carbohydrase in their diet showed similar results as NC + phytase group.

From day 22 to 42, the BWG of broilers fed LNC diets was lower \((p < .01)\) than those fed the PC diet. Birds fed the LNC diet had inferior FCR compared with the PC and NC \((p < .01)\). Dietary supplementation of multi-carbohydrase either into NC or NC + phytase diets improved BWG of broilers compared with NC diet. The BWG reduction was ameliorated when multi-carbohydrase + phytase was supplemented to the LNC diet. The addition of phytase + multi-carbohydrase to LNC diets improved FCR during the finisher phase (22–42 d).

For the entire period, broilers fed either NC or LNC diets had lower \((p < .01)\) body weight (BW) and BWG, and higher \((p < .01)\) FCR than those fed the PC diet (Table 3). The FCR of birds in the LNC group was also higher than those in NC group \((p < .01)\). Supplementation of multi-carbohydrase, phytase and phytase + carbohydrate to NC diet led to similar BWG, FCR, BW, mortality and EPEF in broilers compared with PC group. Supplementation of phytase and multi-carbohydrase nutrient matrix values for amino acids, Ca, available P and AME was reduced (LNC), birds fed multi-carbohydrase in their diet showed similar results as NC + phytase group.

#### Statistical analyses

All data were analysed as a completely randomised design with seven treatments using the General Linear Model procedure of SAS (SAS Institute 2011). The following model was fitted:

\[
Y_{ij} = \mu + T_i + e_{ij}
\]  

Where \(Y_{ij}\) was the trait of interest for chicken, \(\mu\) was the overall mean, \(T_i\) was the treatment effect, and \(e_{ij}\) was the residual error. Normal distribution of residuals and variance homogeneity of the data was tested by the UNIVARIATE procedure and the Levene’s test, respectively. For all the studied traits the pen was used as the experimental unit. Results in tables are reported as means and differences among treatments were compared using the Tukey’s test, and significance was determined at \(p < .05\).

### Table 2. Effect of dietary nutrient reduction and phytase and multi-non-starch polysaccharides enzyme (multi-carbohydrase) supplementation on average body weight gain (BWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of broilers from 1 to 21 d and 22 to 42 d of age.

| Item | 1–21 d | 22–42 d | p-Value |
|------|--------|---------|--------|
|      | BWG, g/d | ADFI, g/d | FCR, g/g | BWG, g/d | ADFI, g/d | FCR, g/g |        |
| PC\(^1\) | 38.400\(^a\) | 54.100 | 1.410\(^b\) | 83.300\(^ab\) | 154.300 | 1.85\(^b\) |        |
| NC\(^2\) | 33.800\(^bc\) | 53.200 | 1.580\(^b\) | 77.800\(^bc\) | 155.000 | 1.99\(^b\) |        |
| NC + phytase\(^3\) | 35.400\(^abc\) | 51.700 | 1.460\(^bc\) | 83.200\(^ab\) | 154.500 | 1.86\(^b\) |        |
| NC + multi-carbohydrase\(^4\) | 37.500\(^ab\) | 54.600 | 1.450\(^bc\) | 84.000\(^a\) | 155.100 | 1.85\(^b\) |        |
| NC + phytase + multi-carbohydrase | 35.800\(^abc\) | 50.800 | 1.420\(^a\) | 84.100\(^a\) | 155.400 | 1.85\(^b\) |        |
| LNC\(^5\) | 32.300\(^c\) | 54.500 | 1.690\(^a\) | 73.500\(^c\) | 159.700 | 2.18\(^a\) |        |
| LNC + phytase + multi-carbohydrase | 34.300\(^c\) | 52.000 | 1.520\(^b\) | 83.000\(^ab\) | 154.400 | 1.86\(^b\) |        |
| SEM\(^\text{n = 4}\) | 0.60 | 1.470 | 0.032 | 1.90 | 2.050 | 0.035 |        |
| p-Value | .001 | .426 | .001 | .001 | .544 | .001 |        |

\(^{a,b,c}\)Means within a column with different superscripts are significantly different \((p < .05)\).

\(^1\)Positive control; a nutritionally adequate diet. \(^2\)Negative control; deficient in nutrients level according to phytase nutrient matrix values for amino acids, Ca, available P and AME. \(^3\)Phytase; Natuphos; 10,000 BASF Corp., Germany; 500 phytase unit/kg diet. \(^4\)Endo-power; Easy Bio Inc., South Korea. The enzyme complex of multi-multi-carbohydrase contained 35 unit/g α-galactosidase activity, 110 unit/g galactomannanase activity, 1500 unit/g xylanase activity and 1100 unit/g β-glucanase activity; 0.01% multi-carbohydrase. \(^5\)Low negative control; deficient in nutrients level according to phytase and multi-multi-carbohydrase nutrient matrix values for amino acids, Ca, available P and AME. \(^6\)Standard error of mean.
Table 3. Effect of dietary nutrient reduction and phytase and multi-non-starch polysaccharides enzyme (multi-carbohydrase) supplementation on average body weight gain (BWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of broilers from 1 to 42 d of age.

| Item | BWG, g/d | ADFI, g/d | FCR, g/g | 42 d-BW, g | Mortality, % | EPEF |
|------|----------|-----------|----------|------------|--------------|-------|
| PC   | 60.800a  | 104       | 1.710c   | 2596b      | 0.750b       | 288a  |
| NC   | 55.800bc | 104       | 1.870b   | 2383bc     | 2.250ab      | 240bc |
| NC + phytase3 | 59.300a | 103       | 1.740b   | 2534ab     | 1.000b       | 277ab |
| NC + multi-multi-carbohydrase4 | 60.700a | 105       | 1.730b   | 2592b      | 1.250ab      | 286ab |
| NC + phytase + multi-multi-carbohydrase | 59.900a | 103       | 1.720b   | 2557b      | 1.000b       | 275ab |
| LNC3 | 52.800c | 107       | 2.030a   | 2261bc     | 2.250b       | 209c  |
| LNC + phytase + multi-multi-carbohydrase | 58.600ab | 103       | 1.760bc  | 2504ab     | 1.500ab      | 271ab |
| SEM4 (n = 4) | 0.760 | 1,460 | 0.026 | 31,780 | 0.378 | 9,960 |

p-Value: .001 .480 .001 .001 .010 .001

a,b,c Means within a column with different superscripts are significantly different (p < .05).

Table 4. Effect of dietary nutrient reduction and phytase and multi-non-starch polysaccharides enzyme (multi-carbohydrase) supplementation on nutrients digestibility of broilers at 21 d of age.

| Item | Dry matter, % | Crude protein, % | AIDE, kcal/g |
|------|---------------|------------------|--------------|
| PC   | 70.300ab      | 79.700bc         | 3214bc       |
| NC   | 68.600bc      | 79.000c          | 3193b        |
| NC + phytase3 | 72.000a | 81.400ab         | 3220bc       |
| NC + multi-multi-carbohydrase4 | 70.800bc | 79.900bc         | 3235ab       |
| NC + phytase + multi-multi-carbohydrase | 72.600b | 81.900a         | 3248a        |
| LNC3 | 65.800c       | 77.000d          | 3110c        |
| LNC + phytase + multi-multi-carbohydrase | 70.200b       | 79.500bc         | 3242a        |
| SEM4 (n = 4) | 0.622 | .001 | .001 | .001 |

p-Value: .001 .001 .001 .001

a,b,c,d Means within a column with different superscripts are significantly different (p < .05).

Digestibility

Broilers fed the LNC diet had lower (p < .01) digestibility of DM and CP, and AIDE than those fed PC diets at 21 d of age (Table 4). Apparent ileal digestibility of DM was similar between PC and NC, while dietary AIDE and ileal digestibility of CP were lower in LNC compared to the NC group. In broilers fed NC diets supplemented with phytase, apparent digestibility of the nutrients was similar to those fed PC diets (p > .05). Phytase + multi-carbohydrase supplementation in NC diets had no effect on DM, CP and energy digestibility. Supplementation of phytase + multi-carbohydrase to LNC diet increased digestibility of nutrients compared to PC group. Broilers fed NC + phytase + multi-carbohydrase had higher CP digestibility than those in PC or NC + multi-carbohydrase group.

Discussion

Performance

The negative effects of reducing nutrients density of diets on broiler performance in the current study are consistent with the findings of Francesch and Geraert (2009) who reported that feeding diets with lower apparent metabolisable energy (AME), amino acid, available P and Ca decreased growth performance of broiler from 1 to 21 and 22 to 42 d of age. The improved performance of broilers fed NC + phytase diet in the current research may be due to the improved utilisation of energy, protein and amino acids. This finding is in agreement with the results of previous studies which reported that performance...
parameters were similar between broilers fed PC diet and those fed NC diet supplemented with phytase (Viveros et al. 2002; Santos et al. 2008; Attia et al. 2019).

There are different economic approaches when considering enzyme incorporation in diet formulation. A simpler and probably more practical application, called ‘on top’, aimed at improving performance, covering the nutrient variation in ingredients and marginal nutrients in the diet, without adjusting their nutritional levels. Another approach is to formulate diets by reducing nutrients and adding exogenous enzymes in order to restore the nutritional value of the standard diet, seeking the same performance that a diet with usual nutritional levels would provide (Perazzo Costa et al. 2008; Pasquini et al. 2017). The use of phytase is now a popular practice in broiler diets, and the interaction between dietary phytate and exogenous phytase has been extensively reviewed in broilers (Selle and Ravindran 2007; Selle et al. 2012; Liu et al. 2015).

In the current study, no difference was observed in broiler growth performance among NC + phytase, NC + phytase + multi-carbohydrase (on top) and LNC + phytase + multi-carbohydrase, showing that the decreased nutritional content of the diet was entirely compensated by increased nutrient utilisation with enzyme supplementation. Multi-carbohydrase supplementation to NC + phytase diet (NC + phytase + multi-carbohydrase) had no more effect than NC + phytase diets on broiler performance. It appears that the less nutrient density in the diet, the greater increase in growth performance could be observed by enzyme supplementation (Adeola and Cowieson 2011). Similar results were reported by Juanpere et al. (2005), Olukosi et al. (2010) and Stefanello et al. (2017) who found that on top supplementation of multi-carbohydrase to C-SBM diets containing phytase had no more beneficial impact on broiler performance. While Jozefiak et al. (2010) and dos Santos et al. (2017) showed improvement in FCR of broilers by on top supplementation of multi-carbohydrase to wheat- and sorghum-SBM diets containing exogenous phytase. In addition, Juanpere et al. (2005) indicated that on top supplementation of multi-carbohydrase to wheat- or barley-SBM diets containing phytase improved growth performance parameters in broilers. The present results, however, contradict the findings of Stefanello et al. (2015) who reported improved growth performance of broilers by on top supplementation of multi-carbohydrase to C-SBM diets supplemented with phytase in high dose (1000 FTU/kg).

Karimi et al. (2013) reported that there was no interaction between the supplementation of phytase and xylanase, suggesting that in C-SBM diets the modes of action of these two enzymes are independent of each other. Moreover, a similar finding was reported by Flores et al. (2017) who showed that supplementation of multi-carbohydrase to C-SBM low energy diets containing phytase had no more beneficial effect than multi-carbohydrase-free diets. Xylanase has been proposed to stimulate a prebiotic effect by increasing concentrations of arabinoxylol- and xylooligosaccharides in the large intestine and caeca, which may increase volatile fatty acid (VFA) production in caeca (Massey-O’Neill, Singh, et al., 2014). However, recently McCafferty et al. (2019) concluded that supplemental xylanase inconsistently influenced the concentration of VFA in broiler’s caeca. The multi-carbohydrase enzyme used in the current experiment contained galactosidase. Oligosaccharides such as x-galactosides are more prevalent in SBM (Knudsen 2014). Dietary supplementation of x-galactosidase could improve utilisation and absorption of nutrients, which could lead to better performance (Pettersson and Pontoppidan 2013). Jasek et al. (2018) reported dietary supplementation of multi-carbohydrase containing x-galactosidase and xylanase improves ileal digestibility of nutrients and amino acids.

Some researchers have indicated that by using multi-carbohydrase and phytase enzyme complexes, it is possible to reduce dietary AME and amino acids that make feed formulation cost-effective, while preserving optimum poultry performance (Cowieson et al. 2006; Francesch and Geraert 2009; Attia et al. 2020). Results from previous studies typically show the need for the use of multi-carbohydrase enzymes in broilers, however, researchers pointed out the need to balance nutritional requirements with the potential capacity of the supplemented enzyme to hydrolyse the cell wall and release the needed nutrients (Adeola and Cowieson 2011). Some studies have also indicated that multi-carbohydrases can increase phytase access to phytate molecules (Olukosi et al. 2007), although the effect of using multi-carbohydrase enzymes in combination with phytase depend on ingredients used in poultry feed. It seems that on top supplementation of multi-carbohydrase enzymes in diets with higher soluble NSP such as wheat- and barley-SBM diets, than higher digestible ingredients, such as C-SBM diets, could have more beneficial effects on broiler performance. Therefore, the findings of the current study indicated that multi-carbohydrase enzyme supplementation to diets based on high digestible
ingredients (C-SBM diets) containing phytase offers a possibility of feed formulation with the least cost in which lower nutrients density provided then resulted in performance comparable to a normal diet.

**Digestibility**

A probable reason for lower nutrients digestibility and AIDE in LNC diet than PC diet may be associated with lower dietary oil content, despite being an energy source, it also tends to minimise the rate of feed passage through the gastrointestinal tract, facilitating better absorption and digestion of nutrients in the diet (Upadhaya et al. 2017). In the current study, apparent absorption and digestion of nutrients in the diet through the gastrointestinal tract, facilitating better absorption and digestion of nutrients in the diet (Upadhaya et al. 2017). In the current study, apparent digestibility of DM and AIDE was similar in broilers fed LNC + phytase + multi-carbohydrase diets compared with those fed on top supplementary multi-carbohydrase to NC + phytase (NC + phytase + multi-carbohydrase). However, the multi-carbohydrase supplementation on top of the NC + phytase diet had no additional effect. Similar results have been reported by Olukosi et al. (2010) who observed that on top supplementation of multi-carbohydrase to C-SBM diets supplemented with phytase had no more beneficial effect on nutrients digestibility and AIDE. In comparison to the results of the current study, Stefanello et al. (2015) reported higher digestibility of DM on top of multi-carbohydrase supplementation to C-SBM diets containing higher phytase dose (1000 FTU/kg), while no difference in CP digestibility and AIDE was shown which confirm our findings. Recently, Woyengo et al. (2019) concluded that a combination of NSPase and phytase (higher dose) supplementation resulted in greater ileal digestibility of GE than independent NSPase and phytase supplementation.

Juanpere et al. (2005) indicated that on top supplementation of multi-carbohydrase increases nutrients digestibility when added to barley-SBM diets containing phytase, although they have not observed more beneficial outcomes when multi-carbohydrase supplemented to phytase containing C-SBM diets. However, the current results are in contrast with the findings of Schramm et al. (2017) who reported that total tract digestibility of DM and energy and ileal digestibility of CP increased by on top supplementation of multi-carbohydrase to C-SBM diets containing phytase. Kocher et al. (2003) observed that the combined addition of pectinase, protease, and amylase increased AMEn when added to C-SBM diets with reduced energy and protein density. The supplementation of enzymes to lower nutrient level diets is recommended to have a greater beneficial impact and to reduce the AME of diets by 3 to 4 percent in feed formulation and thereby have a cost advantage for farmers (Zhou et al. 2009).

In the current research, the addition of multi-carbohydrase to LNC diets demonstrated no additional increase in CP digestibility. As phytase improves the digestibility of the diet, effectively reduces the concentration of undigested amino acids and energy, and it can be expected that there are less undigested nutrients are available for multi-carbohydrase enzyme (Cowieson 2010). Failure to observe increased nitrogen utilisation with the use of multi-carbohydrase enzymes in some studies may be due to intact cell walls, especially when the multi-carbohydrase enzymes do not target the specific bonds shielding the nutrient (Tahir et al. 2008). Moreover, the multi-enzyme used in the current study did not have protease. Due to the relatively low levels of soluble NSPs in C-SBM diets, the ability for multi-carbohydrase preparations to improve performance in such diets is more limited than in wheat- or rye-SBM dependent diets (Karimi et al. 2013). The lack of significant impact of multi-carbohydrase preparation mix on broiler growth performance or increasing the potential of phytase to improve dietary nutrients digestibility indicates that the dietary level of NSPs of the C-SBM diet is lower than the threshold needed to see significant effects of NSPase or their interaction with phytase (Karimi et al. 2013). Therefore, on top supplementation of multi-carbohydrase enzymes to phytase-containing diets tends to have an additional impact only if they contain high NSPs.

**Conclusions**

The findings of the current study confirmed that there were no more beneficial impacts on growth performance parameters, nutrient digestibility and AIDE by adding multi-carbohydrase enzymes on top of phytase-containing C-SBM diets; however, the similar effect observed by reducing the multi-carbohydrase matrix value demonstrating that the matrix of both enzymes can be reduced in broilers’ diet. The results of the current study, therefore, indicate that the use of multi-carbohydrase enzyme-containing α-galactosidase, galacto-mannanase, xylanase and β-glucanase as main activities in phytase-containing C-SBM diets with reduced nutrient density based on the matrix value of both multi-carbohydrase and phytase is a potential approach to least-cost feed formulation, resulting in the same performance that would be given by a diet with normal nutrients levels.
Ethical Approval

All experimental procedures were conducted according to the international protocols and approved by Research Committee of University of Guilan, IRAN.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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