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Abstract

The experiment was conducted to estimate the matrix value of xylanase and the effect of dietary xylanase supplementation on growth performance, viscosity, digestibility, and carcass traits in broiler chickens. A total of 588 one-day-old Ross 308 broiler chicks were raised with a commercial diet until day 7 and seven-day-old chicks were randomly allotted to one of seven dietary treatments with twelve replicates. Diets were corn-soybean meat based with wheat added. Dietary treatments were as follows; four basal diets (PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet) and three different xylanase activity levels diet (NCX-1, 1,500 U/kg xylanase activity; NCX-2, 3,000 U/kg xylanase activity, NCX-3 4,500 U/kg xylanase activity) in the NC-3 diet. The standard xylanase dose was decided from the previous *in vitro* experiment. The weight gain and feed intake were measured and feed efficiency was calculated weekly. One bird per pen was selected and euthanized to harvest the intestinal digesta, breast meat, and leg meat samples on days 24 and 35. The linear and quadratic regression analysis and regression plateau were used to determine the xylanase recommendation and marginal level. The viscosity, digestibility, and proximate analysis of meat were analyzed from taken samples. Xylanase-added treatments were performed for higher (p < 0.05) body weight and body weight gain. Furthermore, xylanase-added treatments showed higher protein digestibility and lower viscosity compared to non-xylanase treatment. The maximum metabolizable energy compensation level of xylanase calculated by the regression was 120 kcal/kg and the marginal xylanase level showed maximum performances were 3,622 U/kg on the linear plateau and 4,000 U/kg on the quadratic plateau. Therefore, our experiment suggested that xylanase addition in an energy deficiency diet not only enhances growth performance but also reduces viscosity, and enhances protein digestibility and the maximum compensation level of metabolizable energy was

120 kcal/kg. The recommended levels of xylanase supplementation were determined to be 3,622 and 4,000 U/kg.

Keywords: broiler, carcass trait, digestibility, growth performance, xylanase

Introduction

The recent unstable international situation makes the price of ingredients used in animal diets fluctuate negatively and those changes increase the overall cost of broiler production. This circumstance makes feed formulators focus on using alternative feed ingredients and feed additives such as enzymes, probiotics, prebiotics, and functional biotics to reduce the feed cost without any disadvantage.

Corn and wheat are majorly used for animal diets, and those contain about 15% soluble and insoluble non-starch polysaccharide (NSP) [1]. Furthermore, arabinoxylans are the major components in NSP which are enriched in plant-originated ingredients. Arabinoxylans act like an anti-nutritional factor that increases viscosity in digesta and decreases feed efficiency and growth performances when they are fed to broilers [2, 3]. Increased viscosity could change the dominant microbiota which might negatively affect digestion and absorption for the host animals [4]. Arabinoxylan polymers also encapsulate the nutrient unavailable by the host animal and it is been estimated about 400 to 450 kcal/kg [5]. Therefore, the supplementation of xylanase in poultry diets has become an essential factor in increasing nutrient utilization by degrading arabinoxylan complexes [6]. Xylanases are generally produced by a plethora of organisms including bacteria, algae, fungi, protozoa, gastropods, and anthropods. Xylanases are glycosidases that catalyze the endohydrolytic of 1,4-β-D-xylosidic linkages in the xylan complex to produce xylose, a primary carbon source for cell metabolism and in plant cell infection by plant pathogens [7]. Xylanases are also known for releasing encapsulated nutrients by degrading xylosidic linkage in the cell wall [8] but some recent studies suggest that degrading the cell wall and releasing nutrients are supported by very limiting *in vivo* experiments [6, 9].

Previous studies about adding xylanases to poultry diets have suggested that the addition of xylanase could improve growth performance, nutrient digestibility, digesta viscosity, and carcass traits [10-14]. However, referred studies suggest different levels of xylanase activity because of the different diet formulations. The matrix value of enzymes presented approximate compensated nutrient levels by degrading and catalyzing with enzymes. Accurately measured matrix values are important when commercial feed formulators are required to use the enzyme. Overestimated matrix values can cause not only depressing performances but also animal welfare and underestimation of the potential loss. Thus, accurate and achievable matrix value estimation in the commercial should be required [15]. Therefore, this experiment was conducted to evaluate the physiological effect and estimate the matrix value of dietary xylanase in the corn-wheat-soybean diet on broiler chickens.

Materials and Methods

All experimental procedures were revised and endorsed by the Animal Care and Use Committee of Chungnam National University, Daejeon, Republic of Korea (Protocol No. 202103A-CNU-062).

Birds and housing

An experiment was conducted using 588 Ross broilers from day 1 to 35 days of age. Seven birds were randomly allotted and raised in each wire floor cage (76 × 61 × 46 cm^3) from day 1 to 7 as an adaptation period, and they were reallocated following similar body weight (134.27 ± 0.950 g; mean ± SEM) and weight distribution on day 8. Each pen was installed with three nipple drinkers and a metal trough. Experimental diets and fresh water were provided *via* nipple drinker and metal trough on an *ad libitum* basis. All the management practices were followed by Ross 308 broiler management guidance (Table 1).

Experimental design and diets

The experiment was conducted using 588 one-day-old Ross 308 broiler chicks for 35 days. All birds were raised with a commercial diet from day 1 to 7 days of age as an adaptation period. On day 8, birds were reallocated to one of seven dietary treatments arranged in a completely randomized design and each treatment had 12 replicate cages. Four basal diets (PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet) were formulated based on corn, wheat, and soybean meal (Tables 2 and 3) to meet or exceed the nutrient requirements of [16], except for energy levels, with two-phase feeding which is the starter (day 8 to 24) and the grower (day 25 to 35) phases. Experimental diets were designed with three different xylanase activity levels (1,500 U/kg, 3,000 U/kg, 4,500 U/kg) in the NC-3 diet to estimate the compensation level of ME. Xylanase activity levels were pre-determined based on a previous study (*in vitro* experiment, unpublished data). All diets contained 0.3% Cr_2O_3 (chromic oxide powder, Daejung Chemicals & Materials Co. Ltd., Siheung, Gyeonggi, Korea) as an index for digestibility analysis on days 24 and 35.

Data collection

Growth performance parameters were measured based on a single pen. Body weight (BW) and feed intake were measured on days 7, 14, 21 24, 28, and 35. Average daily gain, average daily feed intake, and feed conversion ratio were calculated based on measured BW and feed intake every week. Mortality was measured when the dead bird was observed during the experimental period.

Sacrifice bird and sample collection were carried out on days 24 and 35 to estimate the effect of starter and grower phase diets. One bird per cage closer to the median BW of the treatment was selected, stunned by carbon dioxide, and euthanized by cervical dislocation for sample collection.

Meat samples were collected after the bird was sacrificed. Both sides of the pectoralis major and pectoralis minor were collected for breast muscle samples and the right-side drumstick with thigh was collected for leg muscle samples. Dry matter, crude protein, ether extract, and ash were analyzed with the proximate analysis method suggested by AOAC [17].

Ileal digesta samples were collected for analysis of viscosity and ileal nutrient digestibility. 2 g of fresh digesta was collected from digesta and centrifuged (12,000 g, 10 min, 20°C). 0.5 mL supernatant was used for determining viscosity using the viscometer (Brook field DV-III model) at 25°C with a CP40 cone and shear rate of 5-500/s. Before the proximate analysis, digesta are oven-dried at 55°C for 24 h, followed by fine grinding and strained through a sieve of < 0.75 mm. Dry matter, crude protein, ether extract, crude ash, and total gross energy were analyzed with the proximate analysis method suggested by AOAC [17].

Statistical analysis

Data were analyzed as a completely randomized design, using one-way analysis of variance in SPSS software (Version 26; IBM SPSS, Chicago, USA, 2018). A pen is used as the experimental unit for growth performances and the individual bird is used as the experimental unit for digestibility, carcass traits, and viscosity. Tukey's multiple range test was used to compare the significant differences between different pairs of means at ($p < 0.05$) when the data showed a significant difference on ANOVA.

Xylanase assessment

The xylanase was assessed to estimate the compensation amount of ME in an energy-deficiency diet and to evaluate the ideal levels of xylanase in the diet.

The matrix value was estimated following the method suggested by [18]. Orthogonal polynomial contrasts examine responses to ME levels for BW and also xylanase for BW. Linear and quadratic regression were analyzed for xylanase with BW and ME level with BW. The regression equation for the ME levels in the diet and supplemental xylanase level for particular response variables were equated and solved for x.

Equation 1: $Y_m = a_m + b_m x_m$ (linear regression equation for ME)

Equation 2: $Y_x = a_x + b_x x_x$ (linear regression equation for xylanase)

Equation 1 = Equation 2: $a_m + b_m x = a_x + b_x x_x$

Where Y represents the response criterion that is BW, x_m presents ME levels in diets, b_m represents the slope of the response criterion to the dietary energy, and b_x represents the slope of the response criterion to the added xylanase. The linear response equations for ME in diet and that for added xylanase were set to be equal and were solved for BW equivalency values for their respective variable.

The quadratic regression equation is also calculated the same as the linear equation as follows:

Equation 3: $Y_m = a_m + b_m x_m + c_m x_m^2$ (quadratic regression equation for ME)

Equation 4: $Y_x = a_x + b_x x_x + c_x x_x^2$ (quadratic regression equation for xylanase)

Equation 3 = Equation 4: $a_m + b_m x_m + c_m x_m^2 = a_x + b_x x_x + c_x x_x^2$

Matrix values calculated by linear and quadratic regression are compared to check the accuracy of each method.

The marginal xylanase level was estimated by the methods suggested by [19, 20]. The linear and quadratic plateau (broken line) models were used to determine the optimum requirement for dietary xylanase levels. The plateau model consisted of a straight or curvy line with an increasing or decreasing slope and a horizontal line.

The linear model of the one-slope broken line is as follows:

$$Y = a + b (R-x)$$

In these equations, a is the ordinate, R is the abscissa of the breakpoint in the curve, and b is the slope of the line for $x<R$. When $x>=R$, the equation indicates $Y=a$.

The quadratic plateau model is as follows:

$$Y = a + bx + cx^2$$

In the quadratic plateau model, the vertex was regarded as a breakpoint, and x showed over the vertex point, Y was presented as a constant number that the vertex value substituted in the formula. In both models, X and Y indicate the xylanase level and BW. Based on the measured growth performance.

Results

Experimental birds were consuming sufficient drinking water and experimental feed, and no symptoms of death or disease were found due to Sudden death syndrome (SDS) or stress throughout the entire 35-day experiment.

Growth performance

BW, average daily feed intake, average daily gain, and feed conversion ratio from days 7 to 35 were shown in Tables 4 to 7. BW on days 14, 24, and 28 showed significant differences ($p < 0.05$) during the experimental period. Xylanase treatments except for NCX-1 performed a superior BW compared to NC-3 when there was a significant difference ($p < 0.05$). NCX-1 exhibits a significant difference ($p < 0.05$) with NC-3 only on day 24. During all experimental periods, xylanase treatment showed no differences from the PC treatment ($p > 0.05$). Xylanase treatments showed improved ADG ($p < 0.05$) on week 2 compared to NC-3 and there were no differences compared to the PC diet. In the starter, grower, and whole experimental periods, there were no differences in ADG, ADFI, and feed conversion ratio among the treatments ($p > 0.05$).

Carcass traits

Proximate analysis results of breast and leg meat on days 24 and 35 are presented in Tables 8 and 9. There was a significant difference ($p < 0.05$) in the crude protein on leg meat and dry matter content and crude ash on breast meat on day 24. However, xylanase treatments showed no significant differences compared to NC-3 treatment and PC treatment ($p > 0.05$). Furthermore, carcass traits on day 35 have no difference for all measurements ($p > 0.05$).

Viscosity and digestibility

Digesta viscosity and crude protein analysis were presented in Table 10. Xylanase treatment showed significant differences ($p < 0.05$) compared to NC-3 and PC diet on days 24 and 35. Xylanase treatments on ME, DM, and CP showed a significant difference ($p < 0.05$) and performed the same with the PC diet.

Matrix value evaluation

The matrix value estimation with linear and quadratic regression graphs is presented in Tables 11 and 12. The linear regression showed improved matrix value based on the xylanase in the diet increases. On day 14, the matrix value for 1,500 U/kg xylanase was 60.54 kcal/kg, 3,000 U/kg xylanase was 117.12 kcal/kg, and 4,500 U/kg xylanase was 137.53 kcal/kg. On day 24, the matrix value for 1,500 U/kg xylanase was 75.45 kcal/kg, 3,000 U/kg xylanase was 129.69 kcal/kg, and 4,500 U/kg xylanase was 143.77 kcal/kg. On day 28, the matrix value for 1,500

U/kg xylanase was 66.11 kcal/kg, 3,000 U/kg xylanase was 99.32 kcal/kg, and 4,500 U/kg xylanase was 125.41 kcal/kg.

The quadratic regression also showed improved matrix value based on the xylanase increases. On day 14, the matrix value for 1,500 U/kg xylanase was 46.62 kcal/kg, 3,000 U/kg xylanase was 123.22 kcal/kg, and 4,500 U/kg xylanase was 192.38 kcal/kg. On day 24, the matrix value for 1,500 U/kg xylanase was 94.61 kcal/kg, 3,000 U/kg xylanase was 122.88 kcal/kg, and 4,500 U/kg xylanase was 129.21 kcal/kg. On day 28, the matrix value for 1,500 U/kg xylanase was 77.02 kcal/kg, 3,000 U/kg xylanase was 102.53 kcal/kg, and 4,500 U/kg xylanase was 120.36 kcal/kg.

Marginal xylanase amount estimation

Marginal xylanase amount estimation analyzed by linear and quadratic plateau methods was drawn in Figures 1 to 3. The linear plateau indicates the xylanase requirement of 3,500 U/kg and the quadratic plateau indicates the xylanase requirement of 4,400 U/kg on day 14. The linear plateau indicates the xylanase requirement of 3,244 U/kg and the quadratic plateau indicates the xylanase requirement of 3,517 U/kg on day 24. On day 28, The linear plateau indicates the xylanase requirement of 3,622 U/kg and the quadratic plateau indicates the xylanase requirement of 4,000 U/kg.

Discussion

Many previous studies suggest that the xylanase addition in adequate metabolizable energy level diets conditioned with various feed ingredients also presents that xylanase addition also improves growth performances, digestibility, and viscosity [10-12, 21, 22]. The water-soluble arabinoxylans are known as anti-nutritional factors by increasing viscosity and encapsulating the nutrient [23]. The mode of action of xylanase is known that xylanase degrades the arabinoxylan backbone and releases the trapped nutrient. Releasing entrapped nutrients increases the amount of absorption in the small intestine [5, 8]. Broke-down arabinoxylans make the viscosity of digesta lower and it allows the digesta to mix properly and allows the nutrient to be absorbed more easily [24]. Furthermore, small oligosaccharides produced by degrading the arabinoxylan also showed potential prebiotic effects [22, 25]. The viscosity result that a xylanase-added diet exhibits a significantly low viscosity and a higher xylanase-added diet showed a significantly lower viscosity was also supported by previous research.

The exogenous NSP enzymes have been added to improve the nutrient value by degrading the unavailable nutrient complexes and making them available nutrients to the host animal. Especially the absence of xylanase in the poultry intestine makes endogenous xylanase addition in broiler diet experiments conducted continuously. The growth performances of the birds fed xylanase added in energy deficiency diet in the current experiment performed significantly equal to the positive control diet which meets or exceeds the nutrient requirement. Few earlier studies suggest that supplemented enzymes such as xylanase, glucanase, and protease do not affect broiler performances [26-28]. Also, some other research suggests a corn-based diet with xylanase showed no significant difference because corn contains less than 1 g/kg water-soluble NSP [29]. However, those results may be caused by various factors which nutrient levels, diet formulation, enzyme dosage, and bird conditions [30]. On the other hand, numerous studies suggest that exogenous xylanase or NSP enzyme complex addition in a broiler diet could enhance growth performances and ileal digestibility in energy deficiency conditions [31, 32]. Results of xylanase addition in energy deficiency diet propose that the xylanase addition in poultry diet could compensate for the metabolizable energy by degrading the xylan complex or releasing the encapsulated nutrient [5, 8] and following results, the lack of metabolizable energy affect negatively during the whole experimental period and it agreed with the previous xylanase experiment with a reduced metabolizable energy diet [31]. This experiment also exhibits that exogenous xylanase addition could improve growth performances, viscosity, and digestibility compared to the control diet. Therefore, previous studies' results and this experimental result suggest that the birds fed xylanase addition with a calculated energy deficiency diet could be performed equally with birds fed an adequate energy level diet. These results also suggest that the xylanase addition could be a good method to save production costs by reducing metabolizable energy.

The effect of xylanase on poultry meat is controversial with various contrast studies. Some studies suggest that the xylanase addition can improve not only BW gain or final BW but also breast and leg meat weight [33, 34], however, another study suggests the xylanase addition only improves growth performance but carcass traits such as breast muscle yield and relative weight of the abdominal fat pad [35]. These results could be explained that the xylanase addition on the adequate nutrient-contained diet only showed growth performance differences but some basal diet effect on the negative effect so xylanase addition compensates for enough nutrients.

The exact measurement of the amount of xylanase requirement and abilities is very important. Nevertheless, Various previous studies suggest that xylanase could improve performance, but the estimated proper xylanase activities showed diverse levels from 1,250 to 30,000 U/kg [1, 11, 36]. Those big variations of recommended xylanase activity might be caused by the various families of xylanase. Xylanases are classified by displaying varying folds, mechanisms of action, specifications of substrates, different rates, yields, and production of hydrolytic activities, and physicochemical characteristics [37]. Therefore, the individual xylanase requirements should be measured for maximizing animal production and for minimizing enzyme wastage. The methodology for evaluating the optimal level of xylanase follows the previous enzyme study about calculating the equivalent level with a small revision [18]. The compensation level of metabolizable energy and optimal xylanase level showing the maximum compensation level was evaluated by comparing the regression of growth performance and metabolizable energy and the regression of growth performance and xylanase levels. The xylanase matrix value results which 120 kcal compensation on 4,500 U/kg agreed with the previous *in vivo* experiment to estimate the matrix value. The marginal xylanase level was estimated with methods suggested by [19, 20] with a small revision. The linear and quadratic regression formula with a break-point represented the maximum activity level of xylanase and performances. The linear and quadratic plateau regression presents the maximum xylanase of 3,622 U/kg in the linear plateau and the maximum xylanase of 4,000 U/kg in the quadratic plateau on day 28. The compensation levels of experimental results are acceptable when compared to the previous study which added xylanase to the energy deficiency diet [31]. The referred research also suggests that the addition of xylanase in a broiler diet could improve BW and feed conversion ratio.

Conclusion

Xylanase addition in the metabolizable energy deficiency diet could improve growth performance, reduce viscosity, and enhance protein digestibility as much as the common diet by degrading arabinoxylans in the diet. The maximum level of xylanase in the diet was calculated as 4,500 U/kg with 120 kcal/kg metabolizable energy compensation. The marginal levels of xylanase were estimated as 3,622 U/kg with the linear plateau method and estimated as 4,000 U/kg with the quadratic plateau method. These findings suggest that xylanase supplementation could be used for broilers in a metabolizable energy deficiency diet.

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Tables and Figures

Table 1. Seven different dietary treatments of the experimental design.

Treatment	Description
PC	Positive control diet to meet or exceed Ross 308 specification
NC-1	40 kcal/kg ME level reduced from PC diet
NC-2	80 kcal/kg ME level reduced from PC diet
NC-3	120 kcal/kg ME level reduced from PC diet
NCX-1	NC-3 diet with 1,500 U/kg xylanase supplementation
NCX-2	NC-3 diet with 3,000 U/kg xylanase supplementation
NCX-3	NC-3 diet with 4,500 U/kg xylanase supplementation

Table 2. Calculated and analyzed nutrient content and ingredient profiles of the 4 basal diets for the starter phase (as-fed basis, %)

Ingredient	Diet			
	Control	NC-1	NC-2	NC-3
Corn	57.46	56.59	55.71	54.84
Wheat	4.00	5.33	6.67	8.00
Soybean meal (48 %)	30.25	29.89	29.54	29.18
Fish meal	1.90	1.97	2.03	2.10
Vegetable oil	2.35	2.10	1.85	1.60
Limestone	1.08	1.17	1.27	1.36
Mono-calcium phosphate	1.52	1.51	1.51	1.50
Salt	0.30	0.30	0.30	0.30
Vit-Min premix[1]	0.30	0.30	0.30	0.30
Lys-HCl	0.25	0.25	0.25	0.25
DL-Methionine	0.29	0.28	0.28	0.27
Cr_2O_3	0.30	0.30	0.30	0.30
Calculated composition				
Metabolizable energy (kcal/kg)	3,050	3,010	2,970	2,930
Crude protein (%)	21.50	21.50	21.50	21.50
Lysine (%)	1.32	1.32	1.32	1.32
Methionine + Cystine (%)	1.00	1.00	0.99	0.99
Analyzed xylanase activity	None	NCX-1	NCX-2	NCX-3
Xylanase activity (U/g)	-	1.49	3.00	4.52

[1]Vitamin and mineral mixture provided the following nutrients per kg of diet: vitamin A, 24,000 IU; vitamin D3, 6,000 IU; vitamin E, 30 IU; vitamin K, 4 mg; thiamin, 4 mg; riboflavin, 12 mg; pyridoxine, 4 mg; folacine, 2 mg; biotin, 0.03 mg; vitamin B8 0.06 mg; niacin, 90 mg; pantothenic acid, 30 mg; Fe, 80 mg (as FeSO4・H2O); Zn, 80 mg (as ZnSO4・H2O); Mn, 80 mg (as MnSO4・H2O); Co, 0.5 mg (as CoSO4・H2O); Cu, 10 mg (as CuSO4・H2O); Se, 0.2 mg (as Na2SeO3); I, 0.9 mg (as Ca (IO3)・2H2O).

Table 3. Calculated and analyzed nutrient content and ingredient profiles of the 4 basal diets for the finisher phase (as-fed basis, %)

Ingredient	Diet			
	PC	NC-1	NC-2	NC-3
Corn	51.35	52.68	54.02	55.35
Wheat	4.00	4.33	4.67	5.00
Soybean meal (48 %)	27.87	27.60	27.34	27.07
Fish meal	2.20	2.13	2.07	2.00
Vegetable oil	3.30	2.62	1.94	1.26
Corn starch	7.00	6.39	5.77	5.16
Limestone	1.38	1.33	1.28	1.23
Mono-calcium phosphate	1.50	1.50	1.50	1.50
Salt	0.30	0.30	0.30	0.30
Vit-Min premix[1]	0.30	0.30	0.30	0.30
Lys-HCl	0.20	0.21	0.22	0.23
DL-methionine	0.30	0.30	0.30	0.30
Cr_2O_3	0.30	0.30	0.30	0.30
Calculated composition				
Metabolizable energy (kcal/kg)	3,150	3,110	3,070	3,030
Crude protein (%)	20.00	20.00	20.00	20.00
Lysine (%)	1.22	1.22	1.22	1.22
Methionine + Cystine (%)	0.96	0.96	0.97	0.97
Analyzed xylanase activity	None	NCX-1	NCX-2	NCX-3
Xylanase activity (U/g)	-	1.52	3.03	4.49

[1]Vitamin and mineral mixture provided the following nutrients per kg of diet: vitamin A, 24,000 IU; vitamin D3, 6,000 IU; vitamin E, 30 IU; vitamin K, 4 mg; thiamin, 4 mg; riboflavin, 12 mg; pyridoxine, 4 mg; folacine, 2 mg; biotin, 0.03 mg; vitamin B8 0.06 mg; niacin, 90 mg; pantothenic acid, 30 mg; Fe, 80 mg (as FeSO4 ･H2O); Zn, 80 mg (as ZnSO4 ･ H2O); Mn, 80 mg (as MnSO4 ･H2O); Co, 0.5 mg (as CoSO4 ･H2O); Cu, 10 mg (as CuSO4 ･ H2O); Se, 0.2 mg (as Na2SeO3); I, 0.9 mg (as Ca (IO3) ･ 2H2O).

Table 4. Effect of xylanase addition on the body weight of broilers from day 8 to 35 (g)

Treatment[1]	NC-1	NC-2	NC-3	NCX-1	NCX-2	NCX-3	PC	SEM[2]	*p*-value
Day 8	134.24	133.63	134.7	133.89	134.06	134.43	134.96	0.952	0.827
Day 14	348.29^{ab}	348.86^{ab}	331.95^{a}	346.04^{ab}	359.21^{b}	363.96^{b}	359.88^{b}	8.285	0.004
Day 21	635.64	618.30	626.55	651.68	675.88	672.70	657.13	21.989	0.068
Day 24	838.72^{ab}	808.66^{a}	809.55^{a}	843.10^{b}	867.22^{b}	873.48^{b}	862.91^{b}	25.694	0.036
Day 28	1180.82^{abc}	1129.82^{ab}	1120.54^{a}	1168.33^{abc}	1192.33^{bc}	1211.19^{c}	1207.28^{c}	34.989	0.037
Day 35	1703.23	1673.24	1659.35	1726.64	1730.48	1750.60	1725.47	52.052	0.494

[1] PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet; NCX-1, NC-3 diet with 1,500 U/kg xylanase; NCX-2, NC-3 diet with 3,000 U/kg xylanase; NCX-3, NC-3 diet with 4,500 U/kg xylanase

[2] Standard error of means

[a-c] Values in a row with different superscripts differ significantly ($p < 0.05$)

Table 5. Effect of xylanase addition on the average daily gain of broilers from day 8 to 35 (g/d)

Treatment[1]	NC-1	NC-2	NC-3	NCX-1	NCX-2	NCX-3	PC	SEM[2]	*p*-value
Day 8-14	30.72^{ab}	30.96^{b}	28.46^{a}	30.31^{ab}	32.16^{b}	32.79^{b}	32.13^{b}	1.146	0.007
Day 15-21	41.05	38.49	42.09	43.66	45.24	44.11	42.46	2.789	0.279
Day 22-24	67.69	62.46	59.67	63.81	63.78	66.93	68.59	3.425	0.124
Day 25-28	85.53	80.04	77.25	81.31	81.28	84.43	86.09	4.431	0.118
Day 29-35	75.06	77.77	76.97	79.76	76.88	77.06	74.03	4.430	0.909
Day 8-24	42.26	40.47	40.64	41.72	43.13	43.47	42.82	1.500	0.292
Day 25-35	79.68	80.05	78.71	80.32	78.48	79.74	78.41	3.370	0.995
Day 8-35	56.80	56.02	56.88	56.96	57.02	57.72	55.59	1.851	0.942

[1] PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet; NCX-1, NC-3 diet with 1,500 U/kg xylanase; NCX-2, NC-3 diet with 3,000 U/kg xylanase; NCX-3, NC-3 diet with 4,500 U/kg xylanase

[2] Standard error of means

[a-b] Values in a row with different superscripts differ significantly ($p < 0.05$)

Table 6. Effect of xylanase addition on the average daily feed intake of broilers from day 8 to 35 (g/d)

Treatment[1]	NC-1	NC-2	NC-3	NCX-1	NCX-2	NCX-3	PC	SEM[2]	*p*-value
Day 8-14	38.82	38.22	38.31	39.03	40.80	41.37	38.40	0.952	0.498
Day 15-21	66.40	64.86	69.80	72.78	72.59	71.98	63.25	8.285	0.472
Day 22-24	90.67	86.06	83.20	82.95	84.73	88.12	83.76	21.989	0.424
Day 25-28	110.42	104.17	104.55	103.83	103.67	108.5	99.71	25.694	0.378
Day 29-35	108.25	111.17	112.88	111.65	111.05	111.65	97.77	34.989	0.620
Day 8-24	59.33	57.63	59.20	60.67	61.64	62.22	56.64	3.026	0.490
Day 25-35	109.04	108.63	109.85	108.81	108.36	110.50	98.47	6.445	0.560
Day 8-35	78.86	77.66	79.10	79.58	80.00	81.19	73.07	3.765	0.451

[1] PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet; NCX-1, NC-3 diet with 1,500 U/kg xylanase; NCX-2, NC-3 diet with 3,000 U/kg xylanase; NCX-3, NC-3 diet with 4,500 U/kg xylanase

[2] Standard error of means

Table 7. Effect of xylanase addition on the feed conversion ratio of broilers from day 8 to 35 (g/g)

Treatment[1]	NC-1	NC-2	NC-3	NCX-1	NCX-2	NCX-3	PC	SEM[2]	*p*-value
Day 8-14	1.26	1.22	1.30	1.32	1.27	1.27	1.20	0.077	0.737
Day 15-21	1.60	1.64	1.66	1.66	1.60	1.64	1.51	0.107	0.809
Day 22-24	1.31	1.36	1.37	1.33	1.34	1.34	1.24	0.081	0.750
Day 25-28	1.28	1.29	1.33	1.30	1.28	1.30	1.17	0.082	0.575
Day 29-35	1.45	1.44	1.48	1.40	1.44	1.46	1.34	0.089	0.757
Day 8-24	1.41	1.43	1.46	1.46	1.43	1.44	1.33	0.071	0.596
Day 25-35	1.37	1.37	1.40	1.36	1.38	1.40	1.27	0.082	0.719
Day 8-35	1.39	1.39	1.43	1.41	1.40	1.41	1.30	0.072	0.610

[1] PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet; NCX-1, NC-3 diet with 1,500 U/kg xylanase; NCX-2, NC-3 diet with 3,000 U/kg xylanase; NCX-3, NC-3 diet with 4,500 U/kg xylanase

[2] Standard error of means

Table 8. The effect of xylanase addition in the poultry diet on leg meat (%)

Treatment[1]	NC-1	NC-2	NC-3	NCX-1	NCX-2	NCX-3	PC	SEM[2]	*p*-value
Day 24									
Moisture	71.71	72.28	70.34	72.31	72.14	74.38	71.80	1.355	0.208
Crude protein	19.44[ab]	19.95[ab]	19.52[ab]	20.57[b]	19.65[ab]	18.93[a]	19.75[ab]	0.347	0.006
Crude fat	5.26	5.75	5.47	4.56	6.15	5.39	5.51	0.919	0.756
Ash	1.23	1.26	1.33	1.31	1.26	1.15	1.22	0.064	0.158
Day 35									
Moisture	70.06	68.86	68.77	69.56	65.88	67.36	66.57	1.669	0.155
Crude protein	19.64	19.47	19.77	18.73	19.04	18.62	19.32	0.769	0.674
Crude fat	5.03	5.44	6.39	4.29	5.65	4.14	6.95	1.517	0.945
Ash	1.26	1.30	1.19	1.17	1.21	1.20	1.14	0.071	0.320

[1] PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet; NCX-1, NC-3 diet with 1,500 U/kg xylanase; NCX-2, NC-3 diet with 3,000 U/kg xylanase; NCX-3, NC-3 diet with 4,500 U/kg xylanase

[2] Standard error of means

[a-b] Values in a row with different superscripts differ significantly ($p < 0.05$)

Table 9. The effect of xylanase addition in the poultry diet on breast meat (%)

Treatment[1]	NC-1	NC-2	NC-3	NCX-1	NCX-2	NCX-3	PC	SEM[2]	*p*-value
Day 24									
Moisture	73.78^{c}	72.64^{bc}	68.23^{a}	68.57^{ab}	69.23^{ab}	68.30^{ab}	66.92^{a}	1.353	<0.001
Crude protein	24.08	24.30	23.94	24.31	24.52	24.02	24.86	0.471	0.498
Crude fat	1.32	1.25	1.07	0.91	0.98	0.67	1.06	0.212	0.092
Ash	1.60^{ab}	1.53^{ab}	1.68^{ab}	1.79^{b}	1.72^{ab}	1.75^{ab}	1.42^{a}	0.105	0.023
Day 35									
Moisture	68.03	69.18	67.10	67.98	66.28	66.36	66.74	0.991	0.074
Crude protein	21.84	23.70	23.75	23.46	24.50	23.71	23.80	0.894	0.179
Crude fat	1.18	0.75	0.59	1.13	0.77	0.63	1.07	0.281	0.223
Ash	1.66	1.59	1.60	1.63	1.63	1.67	1.64	0.064	0.876

[1] PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet; NCX-1, NC-3 diet with 1,500 U/kg xylanase; NCX-2, NC-3 diet with 3,000 U/kg xylanase; NCX-3, NC-3 diet with 4,500 U/kg xylanase

[2] Standard error of means

[a-c] Values in a row with different superscripts differ significantly ($p < 0.05$)

Table 10. Digesta analysis for viscosity and protein digestibility

Treatment[1]	NC-1	NC-2	NC-3	NCX-1	NCX-2	NCX-3	PC	SEM[2]	*p*-value
Viscosity (mPa/s)									
Day 24	2.76[cde]	2.84[de]	2.93[e]	2.65[abc]	2.55[ab]	2.48[a]	2.73[bcd]	0.061	0.001
Day 35	2.64[cd]	2.78[de]	2.86[e]	2.56[abc]	2.44[ab]	2.38[a]	2.62[bcd]	0.062	0.001
Protein digestibility (%)									
Day 24	81.87[b]	81.87[b]	77.22[a]	79.62[ab]	80.52[ab]	81.52[b]	81.47[b]	0.003	0.011
Day 35	77.62[ab]	77.8[ab]	76.90[a]	82.12[b]	80.75[ab]	80.75[ab]	82.02[ab]	0.009	0.016

[1] PC, energy sufficient diet; NC-1, -40 kcal/kg ME reduced from PC diet; NC-2, -80 kcal/kg ME reduced from PC diet; NC-3. -120 kcal/kg ME reduced from PC diet; NCX-1, NC-3 diet with 1,500 U/kg xylanase; NCX-2, NC-3 diet with 3,000 U/kg xylanase; NCX-3, NC-3 diet with 4,500 U/kg xylanase

[2] Standard error of means

[a-e] Values in a row with different superscripts differ significantly ($p < 0.05$)

Table 11. Xylanase matrix value evaluation by the linear regression graph

Items	Linear regression[1]	R^2	Body weight (g)			Calculated matrix value[2] (kcal/kg)		
			1,500 U/kg	3,000 U/kg	4,500 U/kg	1,500 U/kg	3,000 U/kg	4,500 U/kg
Day 14	331.95+0.23275x	87.162	346.04	359.21	363.96	60.54	117.12	137.53
Day 24	809.55+0.44467x	88.929	843.10	867.22	873.48	75.45	129.69	143.77
Day 28	1120.54+0.72283x	94.289	1168.33	1192.33	1211.19	66.11	99.32	125.41

[1] Regression was drawn with body weight (x) and growth performance (y); x has a range from 0 to 120 kcal/kg, 0 represents the ME level of the NC-3 diet and 120 represents the ME level of the PC diet for convenience

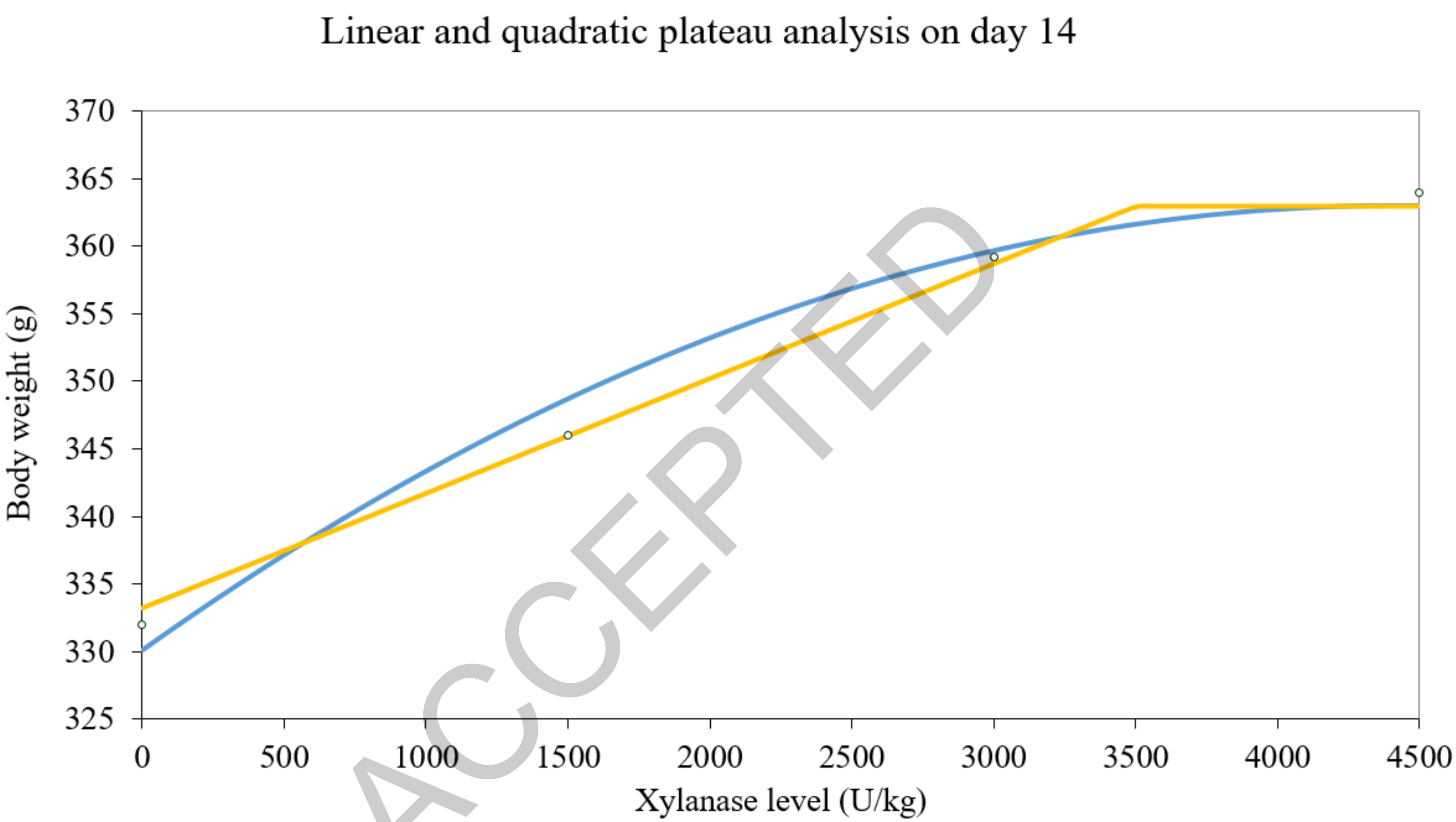
[2] calculated values are calculated by substituting body weight values to estimate the matrix value of xylanase. Estimated values over 120 kcal/kg could be biased

Table 12. Xylanase matrix value evaluation by the quadratic regression graph

Items	Quadratic regression[1]	R^2	Body weight (g)			Calculated matrix value[1] (kcal/kg)		
			1,500 U/kg	3,000 U/kg	4,500 U/kg	1,500 U/kg	3,000 U/kg	4,500 U/kg
Day 14	$-0.0008x^2+0.3078x+333.43$	88.943	346.04	359.21	363.96	46.62	123.22	192.38
Day 24	$0.0039x^2+0.0051x+807.71$	96.665	843.10	867.22	873.48	94.61	122.88	129.21
Day 28	$0.0027x^2+0.4559x+1117.2$	95.726	1168.33	1192.33	1211.19	77.02	102.53	120.36

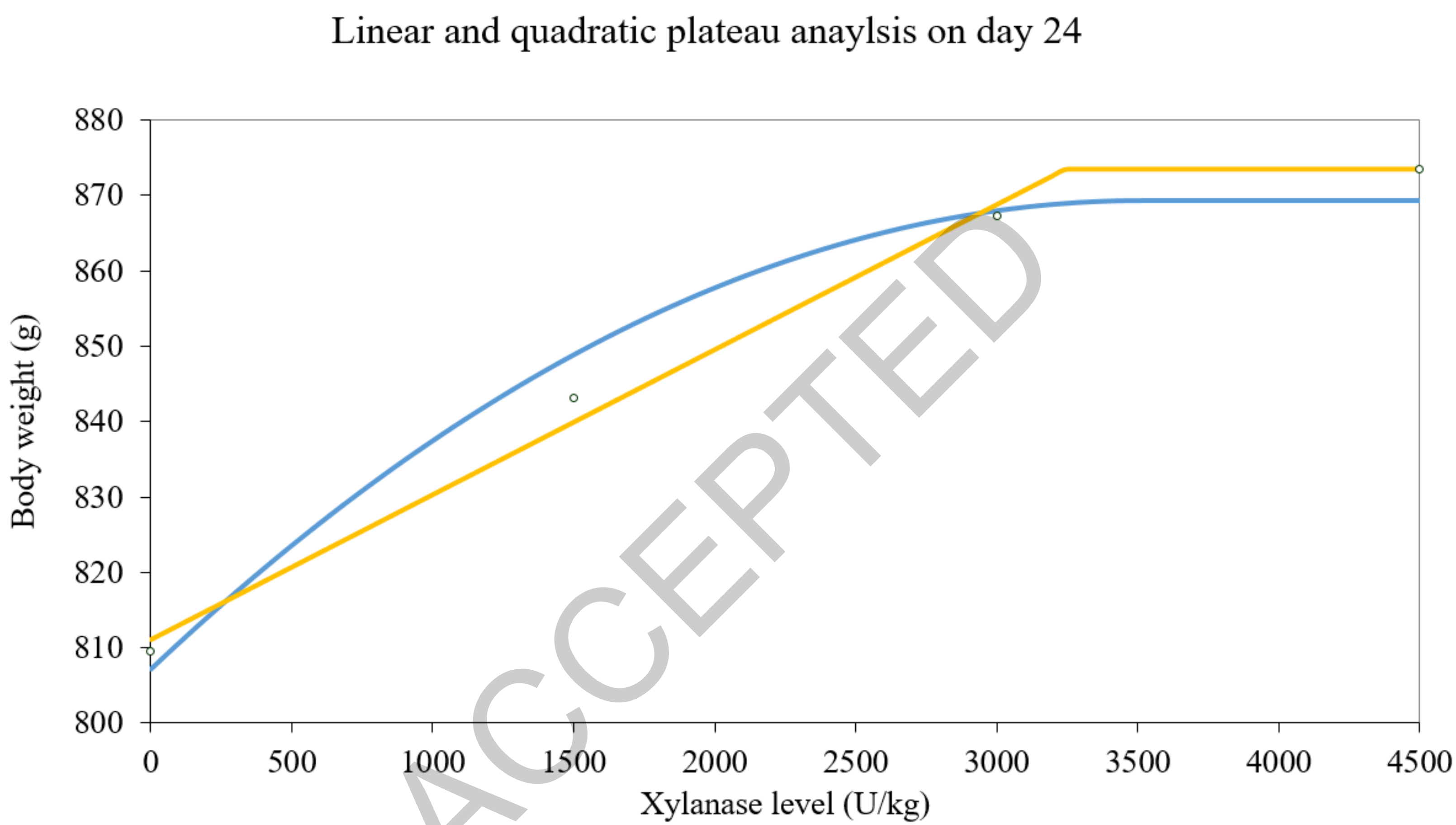
[1] Regression was drawn with body weight (x) and growth performance (y); x has a range from 0 to 120 kcal/kg, 0 represents the ME level of the NC-3 diet and 120 represents the ME level of the PC diet for convenience

[2] calculated values are calculated by substituting body weight values to estimate the matrix value of xylanase. Estimated values over 120 kcal/kg could be biased



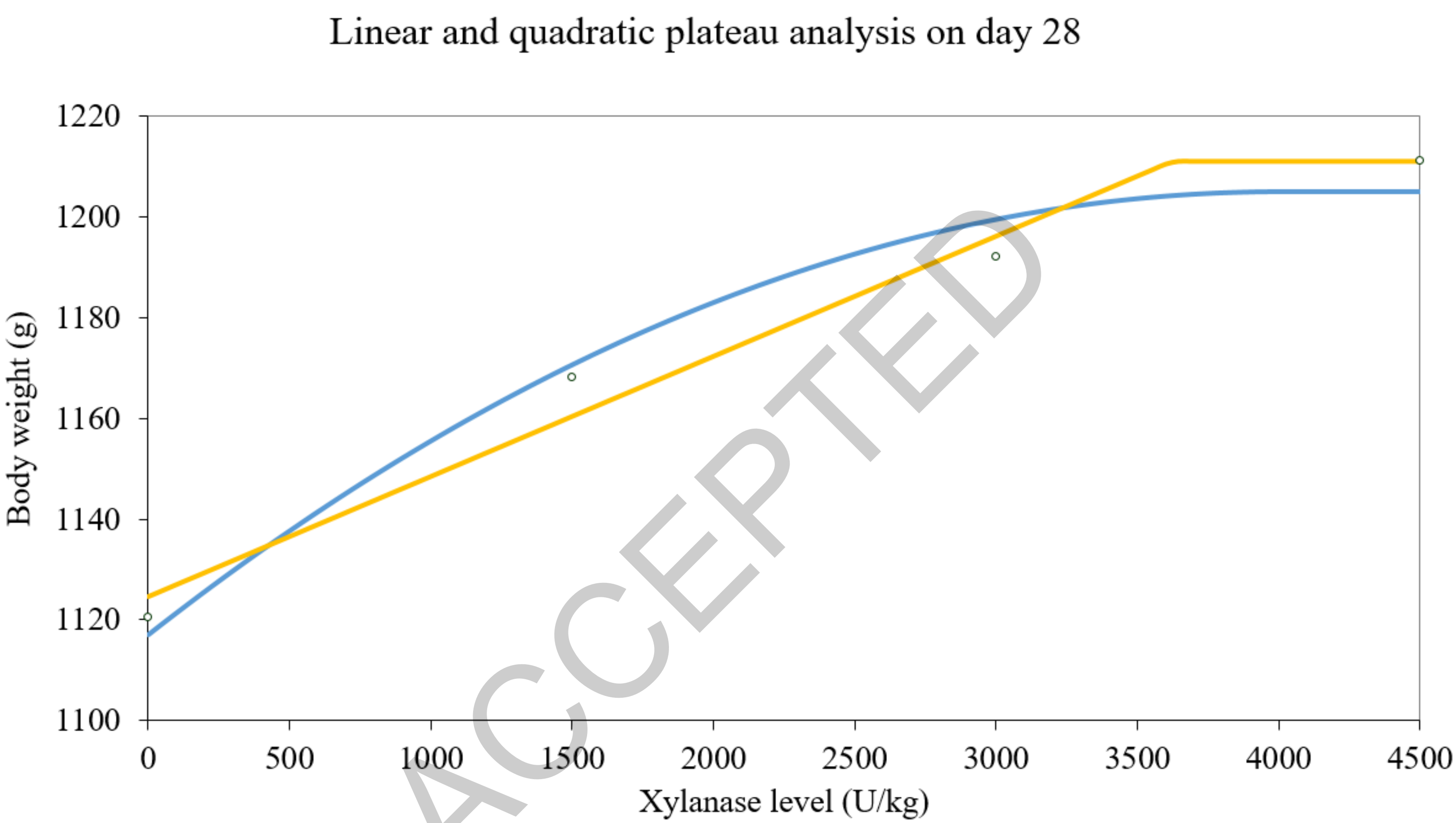
	Requirement (U/kg)	Standard error	P-value	R^2
Linear plateau	3500	1.681	0.003	0.99545
Quadratic plateau	4400	3.319	0.006	0.98117

Figure 1. Linear and quadratic plateau analysis to evaluate the marginal xylanase level on day 14



	Requirement (U/kg)	Standard error	P-value	R^2
Linear plateau	3244	3.850	0.003	0.99412
Quadratic plateau	3517	6.586	0.005	0.97741

Figure 2. Linear and quadratic plateau analysis to evaluate the marginal xylanase level on day 24



	Requirement	Standard error	P-value	R^2
Linear plateau	3622	9.712	0.005	0.97952
Quadratic plateau	4000	9.803	0.005	0.97666

Figure 3. Linear and quadratic plateau analysis to evaluate the marginal xylanase level on day 28