Validation of Microtech phytase in growing broilers under an ad-libitum feeding regime

[1]Juan A. Javierre. [1]Mauricio Delgado, [2]Eddiel Jiménez

[1]Tekzol SAS, [2]Tecnnician

Introduction

A major proportion of phospours content in vegetables used as feed raw materials is found as inositol exakis phosphate, also known as phytic acid or phytin.

This compound is naturally undigestible for the monogastric animals, as they lack the specific enzyme for its degradation. Besides not being an available phosphorous source, phytic acid complexes with a fraction of feed proteins in the lower pH gut segments, and with calcium and potentially other micronutrients in the segments with higher pH level.

Technology evolition provodes the technician with phytase, specific enzymes degrading phytate and releasing the bound phosphorous from vegetable ingredients. The evolution of such enzymes has gone through different stages or generations, each one improving over the previous, covering their deficiencies. Microtech is a third-generation phytase wich characteristics set forth in the following section.

✓ 6-phytase: this kind of enzyme starts degrading phytinon the 6th carbon atom and ends it at carbon number 1. This frees 5 molecules of P_2O_5, while 3-phytases release just 2 molecules.

✓ Recombinant enzyme: the producing microorganism – *Pichia pastoris* - naturally produces a very small amount of other enzymes and abundantly secretes phytase, in almost pure form. It contains the phytase-producing gene, from E. coli, hat has been inserted into its genome.

✓ Glycosilated: *Pichia pastoris* is a N-glycosilated protein producer. This preserves the protein's folding behaviour, which improves the enzyme resistance to thermal stress.

✓ Modified: between 8 and 20 amino acids from the genome sequence have been substituted. This procedure allows altering the three dimensional structure of the enzyme, creating disulphide bridges or hydrogen bonds, for example, that modify the tertiary folding patternr and causing extra protection of the active site. This makes this protein more resistant against gastric acid and proteases, and provide better resistance to pelleting temperatures.

✓ Uncoated: the genetic modifications and the producing source makes unnecessary the coating technologies needed in oher enzymes to meke them surviving the pelleting process of feed. This fesults in earlier catalytic activity upon the phytic acid, increasing its overall efficacy.

With the objective of validating Microtech efficacy under Colombian feeding and rearing conditions, a growing broiler experiment was designed by Tekzol to be run in its Animal Experimentation Station located in Palmira (Valle del Cauca Department).

Aim

The aim for the rearing stage of this experiment was to compare the growing performance of 42 day broiler chickens fed diets with and without Microtech. Feeds were standard corn-soybean meal diets which included dicalcium phosphate (DCP) as complementary phosphorous source (control), or modified to reduce the amount of DCP with the inclusion of 100 ppm Microtech.

The trial's hypothesis was the decrease of phosphorous added to the diet through DCP was compensated by Microtech's activity upon the phytate from the vegetable ingredients and the overall performance of the experimental animals should be, at least, identical to that of the controls.

Experimental design

Animal husbandry

The trial was run in floor pens in Tekzol's Animal Experimentation Unit located in Palmira (Valle del Cauca Department). Broiler density for this trial was set to 8 chickens per square meer.

The birds were reared in a clean and disinfected place, after a proper sanitary void previous to the start of the experiment. New wood shawings were used as litter, at a depth of 10-15 cm. The litter was disinfected with quaternary ammonia and glutaraldehyde disinfectants previous to chick placement.

All feeders were identical in shape and size. They were identified with coloured markers relating the type of feed to receive.

Chicks had feed and water supplied ad libitum throughout the trial.

In each experimental day, the feeders were emptied, the remains weighed (if applicable) and feeders were supplied with fresh feed. Utmost care was exercised to avoid feed spillage during the manipulation of feeders.

The following parameters were surveyed daily: general appearance of the animals; temperature; light; water and feed conditions; litter condition; and mortality. The animals with very poor performance were culled from the trial and the date of culling and weight were recorded for the data adjustement at the end of the trial.

Animals, housing and experimental design

Ross 308 broiler chicks of mixed sexes (50% males and 50% females), coming from a nearby supplier, were used. Animals were distributed at random into treatments and replicates. for this experiment 3 males and 3 females from each replicate were selected and individually marked with colour pactched in certain body segments. These animals were individually controlled during the 6 weeks of the trial.

The experiment's desing responded to a randomized, complete block design (RCBD) with two reatments: a control diet and the same modified in DCP sources and containing 100 ppm Microtech. Every treatment has 4 replications. As indicated previously, the diets were colour-coded on the bags and any particular colour did match the feeder colour, to avoid confusions.

Feeding program

The feeding program has two phases: a starter phase, from 1 to 21 days of age, and a grower phase, from 22 days to 42 days of age Feed was suplied *ad libitum*.

The control diet was a commercial ration based on corn and soybean meal, containing DCP as phosphorous source; the experimental diet was reformulated to achieve the same level of digestible phosphorous while reducing DCP inclusion rates and adding 100 ppm Microtech.

Experimental criteria and treatments

The criterion for the experiment was achieving similar levels of digestible phosphorous taking into account Microtech's ability to release phytate-bound phosphorous. The work hypothesis is to check that the diest conteining Microtech achieve similar productive parameters, at a minimum, when compared with the DCP controls.

Bone mineralization was analysed also as a sign of proper calcium and phosphorous uptake and adequate phytase function.

In Tables 1 and 2 the feed composition and nutritional analysis is shown.

Records

the birs were weighed at the end of aech week, per pen and all those individually tagged. Feed disappearance (apparent consumption) was recorded daily and unconsumed remains were weighed before pouring on fresh feed into the feederes. The average feed intake, average body weight gain and conversion index per pen and period were computed. The individual body weight of all tagged animals was also recorded weekly hrough out the experiment.

Statistical analysis

The data wes subject of analysis of variance (ANOVA) to assess the effect of the experimental diets on the bird performance. The statistical package SPSS was used for the calculations. All declarations of significance were based on a provi-bability level of $p < 0.05$. Averages were separated by means of Duncan Multiple Range test (DMR).

Resultas and discussion

The average feed consumption per pen and growth results by sex and average appear in Tables 3 and 4 and Figures 1 and 2. The values for the Feec Conversion Ratio are shown in Table 5. The experimental design allows the FCR calculations only in pen basis, therefore no statistical analysis is performed on this parameter.

Ingredient	Starter -	Starter +	Grower -	Grower +
Yellow corn	52,1570	60,3324	52,0658	63,5726
soybean meal	19,3094	22,7947	19,2487	20,9849
corn germ meal	8,0000	0,0000	9,7333	0,0000
Full fat soy	10,0000	7,0485	8,5027	4,0000
Corn gluten 60%	2,4526	2,1243	1,0000	3,3477
Meat-bone meal 45%	3,0000	3,0000	3,0000	1,9012
Blood meal	1,2000	1,2000	0,6000	0,6000
Poultry meal	0,0000	0,0000	0,0000	0,2020
Palm oil	1,0000	1,0000	3,5000	2,9397
Limestone	0,4897	0,7087	0,3923	0,8219
DCP	0,9782	0,3214	0,6124	0,1000
Salt	0,1770	0,1815	0,1765	0,1082
Premix	0,5000	0,5000	0,6000	0,6000
Sodium bicerbonate	0,1843	0,1999	0,1535	0,3035
DL-Methionine 99%	0,2361	0,2505	0,1999	0,1880
Lysine HCl	0,2469	0,2499	0,1730	0,2639
Threonine L	0,0689	0,0783	0,0421	0,0563
Microtech	0,00	0,01	0,00	0,01

Table 1. Diet composition as used in the experiment

		Starter -	Starter +	Grower -	Grower +
Moisture	%	11,97	12,40	11,79	12,39
ME poultry	Kcal/kg	3.110,00	3.110,00	3.250,00	3.250,00
Crude protein	%	22,13	21,68	20,40	19,78
Total lysine	%	1,32	1,31	1,16	1,17
Total methionine	%	0,58	0,57	0,51	0,50
Total Met + cis	%	0,95	0,94	0,86	0,84
Total threonine	%	0,89	0,88	0,80	0,78
Total tryptophane	%	0,25	0,24	0,23	0,21
Ether exgtract	%	6,05	5,04	8,37	6,49
Crude fibre	%	3,40	2,78	3,47	2,65
Ash	%	5,19	4,49	4,72	3,85
Calcium	%	0,84	0,86	0,70	0,72
Total P	%	0,70	0,65	0,63	0,57
Available P	%	0,41	0,41	0,34	0,34
Chlorride	%	0,22	0,22	0,20	0,17
Sodium	%	0,17	0,17	0,16	0,16
Linoleic acid	%	2,40	2,03	2,54	2,00

Table 2. Nutritional composition of the diets

Week	Control	Microtech
1	147,50	151,01
2	507,05	515,99
3	1.142,71	1.148,98
4	2.051,17	2.055,76
5	3.197,51	3.172,91
6	4.450,43	4.432,89

Table 3. Feed intake (g) per week

In general, the birds grew with no difficulty and there were no particulr problems duering the course of the experiment until the 6th week. On that time, from the 3d day onwards, a spike in environment temperatures appeared in that caused a condition of acute heat stress to the birds, is spite of the house being fitted with forced ventilation. The net effect was the reduction in growth rate and decreased feed intake, typical symptoms of acute hea stress. Until week 5, the birds receiving feed with Microtech grew to 95% of the standard growing curve for the strain, according to tables provided by the supplier of the genetic (data not shown). The diffferences in the last three days, however,

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Week	Control			Microtech		
	Males	Females	Average	Males	Females	Average
0	38,33	40,75	39,54	41,00	43,25	42,13
1	169,58	175,58	172,58	184,42	185,67	185,04
2	436,75	415,42	426,08	453,92	443,92	448,92
3	856,25	793,75[a]	825,00[c]	900,00	854,17[b]	877,08[d]
4	1.487,50	1.297,92[a]	1.392,71[c]	1.547,92	1.372,92[b]	1.460,42[d]
5	2.189,58	1.816,67[a]	2.003,13	2.243,75	1.931,25[b]	2.087,50
6	2.766,67	2.227,08[a]	2.496,88[c]	2.875,00	2.425,00[b]	2.650,00[d]

Table 4. Average body weight in gram, by sex and treatment, from the birds at the end of each experimental week

a,b-c,d: values in the same row with different exponent are significantly different (P<0.05)

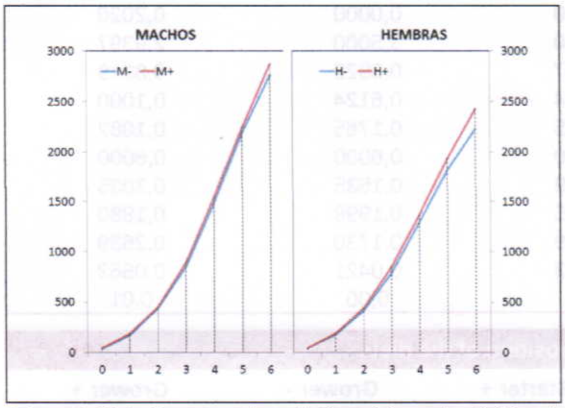

Figure 1. Plot of compared growth (gram) for males (left) and females (right) in 6 weeks with or without Microtech

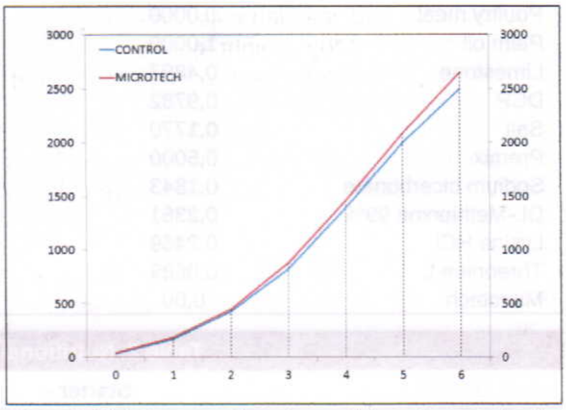

Figure 2. Plot of average 6 week growth (gram) comparing the control or 100 ppm Microtech diets

Week	Control	Microtech
1	0,84	0,84
2	1,16	1,17
3	1,34	1,32
4	1,47	1,45
5	1,58	1,56
6	1,77	1,74

Table 5. Weekly FCR data

were much wider.

In table 4, the weekly growth data for control and experimental animals are shown, split by sex and including the average for each treatment. There were no significant differences between the male weight averages for each week for the control or the 100 ppm Microtech diet.

There were, however, statistically significant differences ($p<0.05$) for the female weight from week 3 onwards, until the end of the experiment. This growth pulse is the responsible for the statistically significant differences between the average data for the control and experimental diets for weeks 3d, 4th, and 6th.

Bone mineralization data

At the end of the experiment, complete hindlegs from control and experimental animals were obtained for determination of the diet effect on the bone mineral status: tibia ash and Ca and P concentration in the ash.

The analysis were carried out in the Laboratory of Nutrition from the faculty of Veterinary Medicine and Animal Science from the National University of Colombia (Bogota Campus). The analytical techniques used were spectrometry for phosphorous and total X-ray reflective fluorescence for calcium. Results are shown in Table 6.

Conclusions

✓ Using 100 ppm Microtech in the diets of growing broiler chickens allows reducing the use of mineral phosphorous sources that are added to feed as a means to reach the mineral requirement

Table 6. Bone mineralization data for the control and 100 ppm Microtech diets		
Parameter	**Control**	**Microtech**
Dry matter, %	90,9	90,7
Ash, %	33,4	33,7
Calcium, %	13,0	13,2
Phosphorous, %	7,4	7,7

for sound growth by the animals.

✓ The diet having the lower content of total phosphorous and supplemented with Microtech allows rates of body weight gain and conversion similar to those receiveing a non-corrected, non-supplemented diet.

✓ There were no differences in the mineral status of bone: neither on total tibia ash nor calcium of phosphorous in the ash.

✓ The diet with the lower content of total phosphorous, supplemented with Microtech costs less than the control diet. At current prices, the average cost of the Microtech diet is 1% cheaper than the control diet.

Take home messages

✓ 100 ppm Microtech added to broiler diets allow reducing the use of mineral phosphorous sources in the feed.

✓ The diets with lower total phosphorpus content supplemented with Microtech achieve similar or better body weight gain and similar FCr than the control birds.

✓ The diets with lower total phosphorpus content supplemented with Microtech are cheaper that the non-supplemented, non-reformulated control dietes.

✓ Microtech permits a profitable broiler production by reducing the diet cost while maintaining the animal's performance.