

Huvepharma NV Uitbreidingstraat 80 2600 Antwerp, Belgium

Tel.: +32 32 88 18 49 Fax: +32 32 89 78 45

customerservice@huvepharma.com

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Information on the use of research results carried out in cooperation with the Department of Poultry Science, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn

Huvepharma EOOD informs that in the period 2017-2019, prof. Krzysztof Kozłowski and colleagues from the Department of Poultry Science, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, conducted research studies on the efficacy of the innovative feed enzyme 6-phytase (OptiPhos® PLUS) in poultry feeding (broiler chickens, laying hens, turkeys). A wider publication of the results of these studies has so far been impossible due to the commercialization process of 6-phytase (OptiPhos® PLUS) as an innovative market product. However, they were used by Huvepharma EOOD, among others, for:

- 1. elaboration of Technical Bulletins (containing short descriptions of experiments and the obtained results, as well as research conclusions), which were and are distributed by the company's employees among poultry and feed producers as informational and promotional materials:
 - OptiPhos® Plus G improves performance, bone ash and ileal digestibility of phytate, phosphorus and protein in a dose-responsive way. Technical Bulletin 2. OptiPhosPlus.TB02.EN02.0520/GI;
 - OptiPhos® Plus G already improves turkey performance at 250 FTU/kg. Technical Bulletin 6.
 OptiPhosPlus.Poultry.TB06.EN02.0520/GI;
 - OptiPhos® Plus G outcompetes OptiPhos® CT in broilers at 500 and 1000 FTU/kg. Technical Bulletin 7. TB.OptiPhosPlus.Poultry7.EN02.0220/GI),
- 2. preparing of documentation necessary in the process of obtaining permission, issued by relevant institutions, for the introduction of 6-phytase (OptiPhos® PLUS) on the market:
 - Scientific opinion of European Food Safety Authority (EFSA). Safety and efficacy of OptiPhos® Plus for poultry species for fattening, minor poultry species reared for breeding and ornamental birds. EFSA Journal 2020;18(6):6141 https://doi.org/10.2903/j.efsa.2020.6141,
 - Scientific opinion of European Food Safety Authority (EFSA). Safety and efficacy of OptiPhos® PLUS (6 phytase) for laying hens, turkeys for breeding, chickens for breeding, minor poultry species for egg production purposes and breeding. EFSA Journal 2020;18(6):6161. https://doi.org/10.2903/j.efsa.2020.6161,
 - Commission Implementing Regulation (EU) 2020/2121 of 16 December 2020 concerning the
 authorisation of a preparation of 6-phytase produced by Komagataella phaffii DSM 32854 as
 a feed additive for all poultry species, ornamental birds, piglets, pigs for fattening, sows and
 minor porcine species for fattening or reproduction (holder of authorisation: Huvepharma
 EOOD) (Official Journal of the European Union, L 426/28, 17 December 2020).

Lode Nollet, MSc., PhD

Global Product Manager Enzymes







OptiPhos® Plus G improves performance, bone ash and ileal digestibility of phytate, phosphorus and protein in a dose-responsive way

Trial description

1 Set-up

- Location: University of Warmia and Mazury, Poland
- Trial period: May June 2018
- Animals: 1666 male Ross 308 broilers distributed over 98 pens
- Feeds (Table 1; pelleted)
 - Starter (d 0-5): 21.5 % CP; 1.1 % dig. Lys; 2985 kCal AME broiler/kg; 0.90 % Ca; 0.45 % aP fed to all treatments
 - Grower (d 5-21): 20.5 % CP; 1.02 % dig. Lys; 3100 kCal AME broiler/kg; 0.60 % Ca; 0.15 % aP
 - Finisher (d 21-35): 19.4 % CP; 0.095 % dig. Lys; 3156 kCal AME broiler/kg; 0.60 % Ca; 0.15 % aP

2 Treatments (only grower and finisher)

- Control feed (as above)
- Control feed with extra Ca (+ 1.5 g) and P as MCP (+ 1.7 aP)
- Control feed with extra Ca (+ 1.5 g) and P as MCP (+ 2.7 g aP)
- Control feed + OptiPhos® Plus G at 250, 500, 750 and 1000 FTU/kg

3 Measurements

- Technical result: growth, feed intake and FCR.
- At day 21: per pen 2 birds were selected of which the right tibia was removed (pooled to one sample) followed by determination of tibia ash on fat free dry matter.
- At day 35: sampling of ileal material from each pen to determine digestibility of P, phytate-P and protein. Samples were taken from the ileum between the diverticulum of Meckel and 10 cm before the ileal/cloacal junction (pooled sample of 3 birds per pen).

Results

- Increasing the levels of MCP or OptiPhos® Plus G increased the final body weight and reduced FCR significantly (Fig. 1). The FCR was already re-established to the level of the P sufficient diet at an inclusion level of 500 FTU OptiPhos® Plus G.
- The supplementation of OptiPhos® Plus G increased bone ash in a dose dependent way, while protein digestibility was increased strongly (> 5 % vs P sufficient controls; Table 2).
- Based on the digestibility data for P and on the bones ash analysis, the calculated equivalency between added doses of OptiPhos® Plus G and dig. P or aP value rose up to 1.53 g dig. P or 2.04 g aP per kg feed at an inclusion of 1000 FTU/kg (Table 3).

Table 1. Feed composition and analysis

| Feed Material | Starter (d 1-5) | Grower (d 6-21) | | | Finisher (d 21-35) | | |
|--------------------------|-----------------|-----------------|-------------------------------------|-------------------------------------|---------------------|-------------------------------------|-------------------------------------|
| | | Control | Control + 1.7 g aP and +1.5 g Ca | Control + 2.7 g aP and +1.5 g Ca | Control | Control + 1.7 g aP and +1.5 g Ca | Control + 2.7 g aP and +1.5 g Ca |
| Corn | 275 | 59 | 280 | 277 | 318 | 302 | 298 |
| Wheat | 300 | 300 | 300 | 300 | 300 | 300 | 300 |
| Soybean meal 49 % CP | 285 | 242 | 245 | 245 | 197 | 200 | 200 |
| Rapeseed meal 33 % CP | 30 | 40 | 40 | 40 | 60 | 60 | 60 |
| Sunflower meal HP | 20 | 30 | 30 | 30 | 30 | 30 | 30 |
| Animal fat | 10 | 40 | 40 | 40 | 40 | 40 | 40 |
| Soybean oil | 38.0 | 23.0 | 28.5 | 30.0 | 29.0 | 34.5 | 36.0 |
| Limestone | 14.5 | 13.6 | 14.0 | 11.9 | 12.0 | 12.9 | 10.7 |
| MCP | 14.2 | 1.0 | 8.6 | 13.0 | 1.2 | 7.8 | 12.3 |
| Others* | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Nutritional value (g/kg) | | | | | | | |
| Crude protein (g/kg) | 216 | 204 | 204 | 204 | 191 | 191 | 190 |
| Crude ash (g/kg) | 62 | 47 | 57 | 55 | 44 | 52 | 54 |
| Dig. lysine (g/kg) | 11.0 | 10.2 | 10.2 | 10.2 | 9.5 | 9.5 | 9.5 |
| Calcium (g/kg) | 9.0 | 6.5 | 8.0 | 8.0 | 6.0 | 7.5 | 7.5 |
| Av. Phosphorus (g/kg) | 4.5 | 1.5 | 3.2 | 4.2 | 1.5 | 3.0 | 4.0 |
| Total P (g/kg) | 7.2 | 4.2 | 5.9 | 6.9 | 4.2 | 5.7 | 6.7 |
| ME (kcal/kg) | 2985 | 3110 | 3109 | 3109 | 3156 | 3156 | 3156 |

^{*} Salt, Sodium Bicarbonate, Synthetic Amino Acids and vitamin/mineral premix

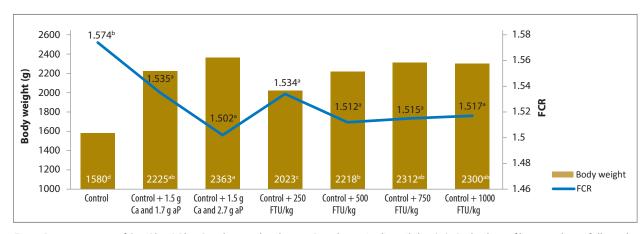


Fig. 1. Doses response of OptiPhos® Plus G on bone ash, phytate-P and protein digestibility (%); (a,d) values of line or column followed by different letter are sign. different (p < 0.05))

Table 2. Doses response of OptiPhos® Plus G on bone ash and protein digestibility (%)

| Bone ash | Protein digestibility |
|-------------------|--|
| 36.9° | 69.8° |
| 47.9ª | 73.5 ^b |
| 45.7⁵ | 74.4 ^b |
| 40.1 ^d | 78.9ª |
| 42.8° | 79.2ª |
| 43.8° | 77.8ª |
| 45.9 ^b | 77.6ª |
| | 36.9° 47.9° 45.7° 40.1° 42.8° 43.8° |

a,c: values in a column with different superscripts are sign. different (p< 0.05)

Table 3. Improvement on dig. P (ileal digestibility analysis) or on aP (bone ash analysis) by increasing levels of OptiPhos® Plus G

| Treatment | Dig. P (g/kg) | P as MCP (g/kg) |
|--------------------------|---------------|-----------------|
| OptiPhos Plus @ 250 FTU | 1.00 | 0.66 |
| OptiPhos Plus @ 500 FTU | 1.12 | 1.30 |
| OptiPhos Plus @ 750 FTU | 1.54 | 1.54 |
| OptiPhos Plus @ 1000 FTU | 1.53 | 2.04 |

Conclusion

- Increasing levels of OptiPhos® Plus G led to increased degradation of phytate-P, yielding a dig. P value of 1.53 g and an aP value of 2.04 g at 1000 FTU/kg inclusion level.
- Protein digestibility is enhanced up to 5 % by increasing levels of OptiPhos® Plus G. This equals 1 % protein per kg feed savings assuming a feed protein level of 20 %.