

# Invited Speakers

## S1.3

### Phosphorus Utilisation in Broilers

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Pollution relative to phosphorus excretion in poultry manure but also the soaring prices of phosphate, a non-renewable resource, remain of major importance in monogastric animal feeding. Given these concerns, the optimisation of phosphorus utilisation by poultry appears crucial for a sustainable production. The use of microbial phytase represents one of the strategies developed during last decades to answer this question. In this context and in order to maintain performance, the nutritionists have to manage adequately the reduction in phosphorus inputs in feed containing exogenous phytase, in relation with other components such as calcium. To highlight this approach, a meta-analysis was performed on starter broilers to quantify the effects of the main dietary interfering factors, microbial phytase and calcium, on phosphorus utilisation and to determine in which conditions chick's growth performance and bone mineralisation are optimised.

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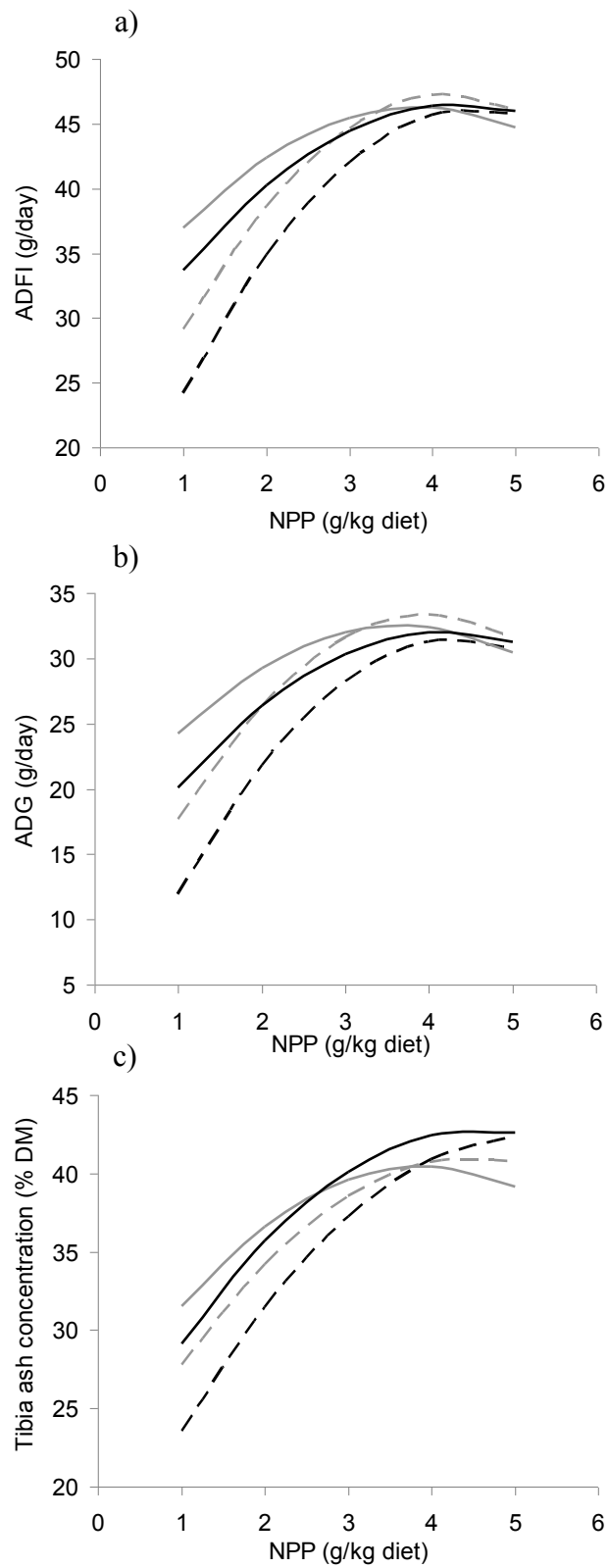
Keywords: phosphorus; calcium; microbial phytase; chicks

## Introduction

Optimisation of phosphorus (P) supply in animal feed remains a major environmental and economical challenge. The poor utilisation of phytic P from feedstuffs by monogastric animals implies a costly mineral feed supply and generates high P excretion. Soil accumulation of phosphate in areas of intensive production represents a source of pollution with deleterious impacts on surface water quality (e.g. eutrophication). Also, animal feed production is dependent on P derived from phosphate rock, which is a non-renewable resource. In this context, nutritional strategies have been developed in chicks to limit P overfeeding and control P excretion. They rely on a better adjustment of P supply to requirement and improved P availability resulting from the introduction of exogenous phytases that are recognised as a reliable alternative source of P in poultry feed (Selle and Ravindran, 2007). Nevertheless, considering the critical role of P in the maintenance of chick's growth performance and bone mineralisation (Underwood and Suttle, 1999), these approaches require a precise knowledge of animal requirements.

Current dietary P recommendations (INRA, 1989, NRC, 1994) are based on optimising bone mineralisation. Overall P utilisation is governed by digestive and metabolic phenomena, in which several dietary components such as calcium (Ca) and phytase interact. Indeed, even if Ca remains essential for bone tissue development, it is important to keep in mind that in low P diets, high dietary Ca deteriorates growth performance and bone mineralisation (Nelson *et al.*, 1965) and could also interact with phytase efficiency at the digestive level (Sebastian *et al.*, 1996, Qian *et al.*, 1997). A better understanding of animal responses to dietary P in interaction with these main interfering factors, thus, represents a pre-requisite when drawing up new recommendations. A difficulty inherent to poultry is the poor possibility to explore the digestive sphere given the mixing of faeces and urine. Digestive phenomena related to phytase and Ca cannot be identified precisely and P utilisation evaluation implies the use of global criteria such as growth performance and bone mineralisation that combine digestive and metabolic responses.

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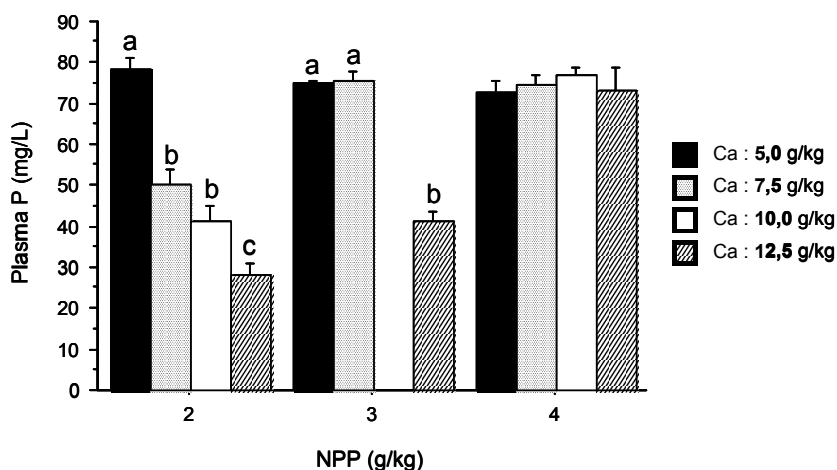
**Figure 1 Response of (a) Average Daily Feed Intake (ADFI), (b) Average Daily Gain (ADG) and (c) tibia ash concentration to dietary NPP (g/kg diet) in chicks given diets with different Ca concentrations (in black, 10 g Ca/kg diet; in grey, 6 g Ca/kg diet) and phytase activity (dashed curve, 0 FTU/kg diet; continuous curve, 500 FTU/kg diet). (Curves fitted from data of Mitchell and Edwards, 1996a, Mitchell and Edwards, 1996b, Sebastian *et al.*, 1996, Denbow *et al.*, 1998, Waldroup *et al.*, 2000, Yan *et al.*, 2003, Dilger *et al.*, 2004, Pillai *et al.*, 2006).**

## Calcium and microbial phytase modulate the effects of non phytic phosphorus on chick performance

Considering the large number of publications dealing with the effects of Ca and microbial phytase (from *A. niger*) on P utilisation in chicks raised until 21 days of age and fed diets based on maize and soybean meal, a meta-analysis was performed in order to quantify the impact and interactions of these major dietary components on chicks performance (Sauvant *et al.*, 2008). Animal responses to non-phytic P (NPP) in terms of growth performance (average daily feed intake: ADFI; average daily gain: ADG) and bone mineralisation (tibia ash concentration), are reported at various level of Ca and microbial phytase supply (Figure 1). The NPP value was calculated as the difference between total P and phytic P estimated from the table of feedstuffs composition of INRA-AFZ (INRA-AFZ, 2004). Dietary phytic P concentration was fairly constant ( $2.30 \pm 0.22$  g/kg diet).

### IMPACT OF DIETARY CALCIUM

As shown in Figure 1, the results of the meta-analysis clearly illustrate the deleterious effect of P deficiency on average daily feed intake (ADFI), average daily gain (ADG) and bone ash concentration. In chicks, the loss of appetite related to P deficiency has previously been reported (Underwood and Suttle, 1999) and, together with depressed feed efficiency, results in depressed growth rate. The major role of P on skeleton formation and cell metabolism clearly explains why its deficiency affects chick growth. In addition, high dietary Ca aggravated P deficiency as shown by depressed ADFI, ADG and tibia ash concentration. The meta-analysis reveals that, in diets without added phytase, when dietary Ca increased from 6 to 10 g/kg in chicks fed 3.5 g NPP/kg, ADFI, ADG and tibia ash concentration were reduced by 2.1 g/day, 2.7 g/day and 0.5 unit respectively, whereas with 1.5 g NPP/kg, they were depressed by 4.4 g/day, 5.2 g/day and 3.4 units respectively. This interaction between Ca and P is a well known phenomenon (Nelson *et al.*, 1965) that has been observed in previous studies presenting a multifactor design regarding dietary Ca and P (Driver *et al.*, 2005, Rama Rao *et al.*, 2006) but has never been fully quantified.



**Figure 2 Plasma concentration of phosphorus (mg/L) in chicks fed maize-soybean meal based diets containing 2, 3 and 4 g NPP/kg and 5.0, 7.5, 10.0 and 12.5 g Ca/kg from 4 to 21 days of age. Values are means  $\pm$  SE (n = 8), within dietary NPP level, means without a common letter differ ( $P < 0.001$ ).**

Calcium was reported to reduce digestive utilisation of P by decreasing the amount of P in an absorbable form through the formation of calcium phosphate insoluble precipitates ( $\text{Ca}_2\text{PO}_4$ ) (Hurwitz and Bar, 1971). As reported in a trial conducted in our lab combining 3 and 4 dietary concentrations of NPP and Ca, respectively, the decrease in absorbed P flow related to low P-high Ca diets is accompanied by a drop in phosphoremia (Ca x NPP,  $P < 0.001$ ) that may mediate the loss of appetite (Figure 2).

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Current recommendations for chicks up to 21 days of age state that 10 g Ca and 4.2 g available P (INRA, 1989) or 4.5 g non-phytate P (NRC, 1994) per kg diet meet requirements. In similar conditions of Ca supply (10 g Ca/kg), the meta-analysis (Figure 1) indicates that in diets containing 10 g Ca/kg, 4.5 and 4.4 g NPP/kg are needed to maximise ADFI and ADG respectively, whereas similar performance can be achieved with only 3.4 and 3.0 g NPP/kg if Ca is lowered to 6 g/kg. These results indicate clearly that it is possible to spare NPP if Ca is concomitantly decreased. Rama Rao *et al.* (2006) have also observed similar body weight gains and feed intakes in chicks given diets containing 9 g Ca and 4.5 g NPP/kg compared to 6 g Ca and 3 g NPP/kg from 2 to 14 days of age.

Studies performed to assess the interaction between dietary Ca and P have generally focused on the Ca:P ratio but did not consider that the interaction between Ca and P firstly depends on the amount of dietary P and change with its level. Nevertheless, the models (Figure 1) challenge the relevance of the use of a fixed Ca:P ratio for all dietary P levels since the Ca:NPP ratio maximising ADG and tibia ash concentration depends on NPP level. For both criteria, the lower NPP is, the lower the Ca:NPP ratio ensuring the maximum response is. For each dietary NPP supply considered, Ca has to be adjusted to a level that optimises P utilisation, with an adequate compromise between growth performance and bone mineralisation.

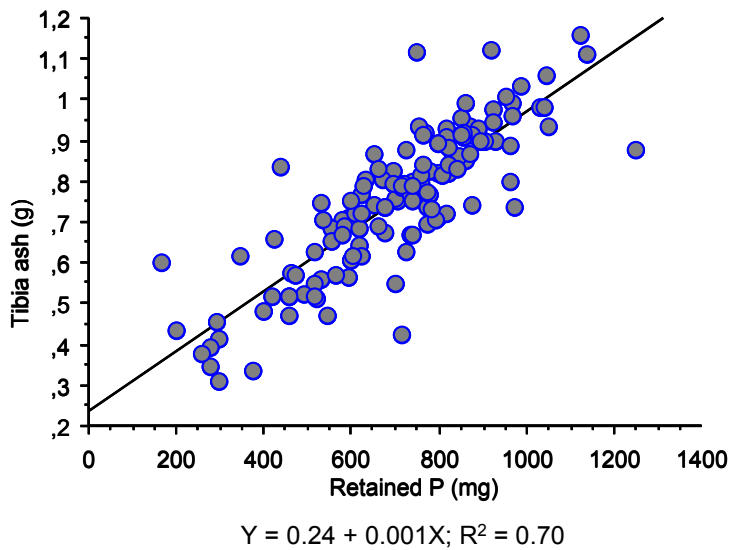
## THE ROLE OF PHYTASE

The models of the meta-analysis (Figure 1) show that bird response to added phytase in terms of ADFI, ADG and tibia ash concentration was exacerbated when decreasing dietary NPP. More precisely, the addition of 500 FTU phytase/kg increased ADFI, ADG and tibia ash concentration by 4, 4 and 8% respectively in broilers fed a diet containing 3.0 g NPP/kg, whereas in those fed 1.5 g of dietary NPP/kg, these parameters were increased by 20, 27 and 18% respectively. Thus, the lower NPP is and the higher Ca is, the greater P deficiency is, explaining the greater bird response to additional P supply, whatever its origin (through addition of NPP or phytase) as previously mentioned by Driver *et al.* (2005). It is also important to underline that P delivery by phytase could remain insufficient to meet requirements when P deficiency is pronounced due to the limited and relatively stable amount of dietary phytate (around 2.3 g/kg). For example, although the addition of microbial phytase (500 FTU/kg) induced an increase in ADG from 25.5 to 28.7 g/day with 2.5 g NPP/kg and 10 g Ca/kg, ADG remains 10% lower than the maximal response. The present models also confirm the inaccuracy of the hypothesis that high Ca:P ratios in P-deficient diets depress bird response to P delivered by phytase (Sebastian *et al.*, 1996, Qian *et al.*, 1997). As previously pointed out by Driver *et al.* (2005), it is important to make the distinction between the concentration of Ca and P at which phytase addition results in maximum performance and that at which animal response to phytase was the greatest. For example, in the present models, birds that received diets containing 2 g NPP/kg and 500 FTU phytase/kg, performed better with 6 g Ca/kg than with 10 g Ca/kg (ADG: 29.3 vs 26.4 g/day). Nevertheless, the magnitude of the response to added phytase in terms of ADG was lower in birds fed 6 g/kg (+11%) than those fed 10 g Ca/kg (+21%).

## Growth performance vs bone mineralisation relative response

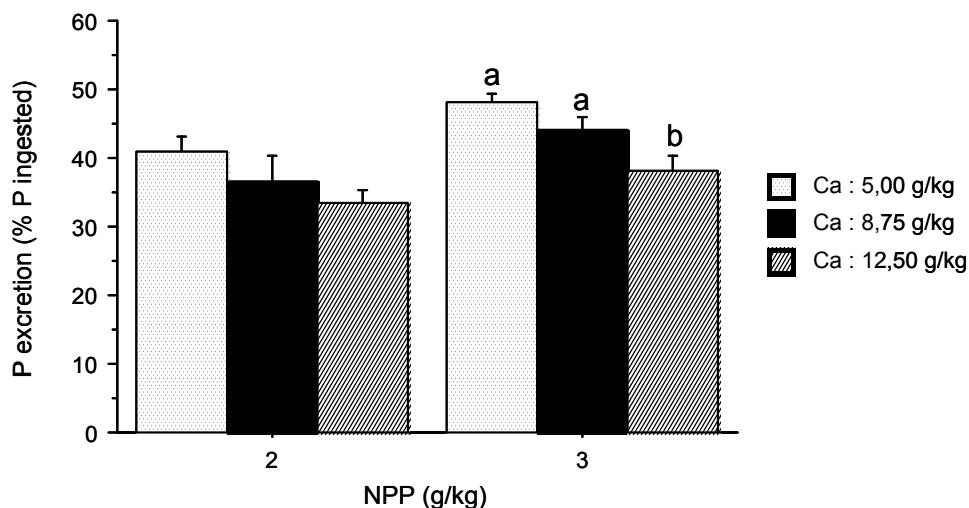
The models issued from the meta analysis (Figure 1) confirm that the maximisation of tibia mineralisation requires higher dietary NPP than those optimising growth performance criteria: in chicks fed 10 g Ca/kg diets, more than 5.0 vs 4.4 g NPP/kg are needed for maximising tibia ash concentration vs ADG respectively, whereas with 6 g Ca/kg, 4.2 vs 3.0 g NPP/kg is needed. A higher P requirement for bone mineralisation than for soft tissue growth has previously been reported (Sauveur, 1985). This is not surprising since most body P is fixed in bones (~85%) as illustrated by the meaningful correlation between retained P (balance trial performed in our lab) and tibia ash weight in 21-day old chicks fed diets with different concentrations of NPP and Ca (Figure 3). Furthermore, almost all body Ca (95%) combines with P to form hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), the mineral phase of bones. Thus, maximal bone response required more P but also more Ca than maximal growth response.

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**Figure 3 Comparison between tibia ash weight (g) and retained phosphorus (mg) during the 5 days prior to slaughter (balance trial performed between 17 and 21 days of age) in chicks fed diets containing different amounts of phosphorus and calcium supplemented or not with microbial phytase until 21 days of age. Each point represents one observation.**

In this context, although reducing dietary Ca may promote growth performance at low dietary P, its decrease must be carried out with care as it could in turn limit bone mineralisation. In phytase supplemented diets, birds fed diets containing 6 g Ca/kg and above 2.5-3.0 g NPP/kg show a depressed tibia ash concentration compared to those fed diets with 10 g Ca/kg (Figure 1). It has previously been shown in chicks that lowering dietary Ca from 8.3 to 5.3 g/kg in diets containing 3.5 g NPP/kg, enhanced growth performance, but depressed tibia dry matter (5%) and ash weight (7%) (Létourneau-Montminy *et al.*, 2008). In the same study, when lowering dietary Ca, tibia ash deposited per g of Ca intake increased by 34%, whereas ash deposition per g of P intake decreased by 14%. Furthermore, Al-Masri *et al.* (1995) have shown that as the decrease in dietary Ca stimulates P absorption, urinary losses of P were concomitantly increased. These results confirm that the decrease in dietary Ca must be performed with caution since the amount of P not fixed in bone is eliminated through urine which is unsatisfactory from an environmental point of view. As reported in Figure 4, P excretion (% ingested) in chicks fed maize and soybean meal based diets supplemented with 500 FTU of microbial phytase per kg depends on dietary Ca concentration ( $P < 0.01$ ). With diets containing 12.50 g Ca/kg, the proportion of ingested P excreted by chicks is lower than with 8.75 (35.7 vs 40.3%) and 5.00 (35.7 vs 44.4%) g Ca/kg.



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**Figure 4 Phosphorus excretion (% ingested phosphorus) in chicks fed diets based on maize and soybean meal supplemented with microbial phytase (500 FTU/kg) containing 2 and 3 NPP/kg and 5.0, 8.75 and 12.5 g Ca/kg from 4 to 21 days of age. Values are means  $\pm$  SE (n = 12), within dietary NPP level, means without a common letter differ ( $P < 0.001$ ).**

Similarly, chick response to additional P depends on the criteria considered since the P requirement differs between soft and bone tissue (Lima *et al.*, 1997). It is generally assumed that body weight gain does not represent a sensitive criterion to evaluate Ca and P adequacy, unlike bone ash. From the models of the meta-analysis (Figure 1), animal response to additional P, provided notably by phytase, was strongly depending on dietary Ca and NPP supply for growth performance. In contrast, it appeared fairly constant when considering tibia ash concentration. This confirms the relevancy of assessing sources of NPP, including phytase, by means of bone mineralisation indicators rather than those measuring growth performance, in conditions of P and Ca supply that do not allow maximisation of bone ash concentration (Yoshida and Hoshii, 1977). Thus, a balance must be found so that levels of Ca necessary for bone mineralisation are not detrimental to growth performance. In this way, a multicriteria approach should be applied.

## Conclusion

This work showed that it is possible to spare dietary P without impairing performance by concomitantly reducing dietary Ca supply. Nevertheless, when dietary P supply is low, increase in dietary Ca affected negatively feed intake inducing a meaningful fall in growth. These results underline the need to monitor carefully dietary Ca supply, notably in the current economical and environmental context that leads to decreased dietary P. Moreover, it is noteworthy that dietary Ca decrease must be performed cautiously to ensure a satisfactory bone mineralisation. Also, since bird response to additional P depends greatly on dietary P and Ca levels and on response criteria, this work confirms the need to evaluate P sources, including phytase, in standardized conditions. In particular, considering the large variation of animal response in term of growth performance, bone mineralisation criteria should be preferred. It would be very useful to extend this analysis to the finishing period when broilers eat two thirds of the food.

## References

- DENBOW, D.M., GRABAU, E.A., LACY, G.H., KORNEGAY, E.T., RUSSELL, D.R. and UMBECK, P.F.** (1998) Soybeans transformed with a fungal phytase gene improve phosphorus availability for broilers. *Poultry Science* **77**: 878-881.
- DILGER, R.N., ONYANGO, E.M., SANDS, J.S. and ADEOLA, O.** (2004) Evaluation of microbial phytase in broiler diets. *Poultry Science* **83**: 962-970.
- DRIVER, J.P., PESTI, G.M., BAKALLI, R.I. and EDWARDS, J.H.M.** (2005) Effects of calcium and nonphytate phosphorus concentrations on phytase efficacy in broiler chicks. *Poultry Science* **84**: 1406-1417.
- HURWITZ, S. and BAR, A.** (1971) Calcium and phosphorus interrelationships in the intestine of the fowl. *Journal of Nutrition* **101**: 677-686.
- LÉTOURNEAU-MONTMINY, M.P., LESCOAT, P., NARCY, A., SAUVANT, D., BERNIER, J.F., MAGNIN, M., POMAR, C., NYS, Y. and JONDREVILLE, C.** (2008) Effect of reduced dietary calcium and phytase supplementation on calcium and phosphorus utilisation in broilers with modified mineral status. *British Poultry Science* **49**: 705-715.
- MITCHELL, R.D. and EDWARDS, H.M.** (1996a) Additive effects of 1,25-dihydroxycholecalciferol and phytase on phytate phosphorus utilization and related parameters in broiler chickens. *Poultry Science* **75**: 111-119.
- MITCHELL, R.D. and EDWARDS, H.M.** (1996b) Effects of phytase and 1,25-dihydroxycholecalciferol on phytate utilization and the quantitative requirements for calcium and phosphorus in young broiler chickens. *Poultry Science* **75**: 95-110.
- NELSON, T.S., HARGUS, W.A., STORER, N. and WALKER, A.C.** (1965) The influences of calcium and phosphorus utilisation in chicks. *Poultry Science* **44**: 1508-1513.
- PILLAI, P.B., O'CONNOR-DENNIE, T., OWENS, C.M. and EMMERT, J.L.** (2006) Efficacy of an *Escherichia coli* phytase in broilers fed adequate or reduced phosphorus diets and its effect on carcass characteristics. *Poult Sci* **85**: 1737-45.
- QIAN, H., KORNEGAY, E.T. and DENBOW, D.M.** (1997) Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:Total phosphorus ratio in broilers diets. *Poultry Science* **76**: 37-46.
- RAMA RAO, S.V., RAJU, M.V.L.N., REDDY, M.R. and PAVANI, P.** (2006) Interaction between dietary calcium and non-phytate phosphorus levels on growth, bone mineralization and mineral excretion in commercial broilers. *Animal Feed Science and Technology* **131**: 133-148.
- SAUVANT, D., SCHMIDELY, P., DAUDIN, J.J. and ST-PIERRE, N.** (2008) Meta-analyses of experimental data: Application in animal nutrition. *Animal*
- SAUVEUR, B.** (1985) Besoins en minéraux et recommandations d'apport. *Rev. Alim. Anim.* **389**: 46-48.

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**SEBASTIAN, S., TOUCHBURN, S.P., CHAVEZ, E.R. and LAGUE, P.C.** (1996) Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. *Poultry Science* **75**: 1516-1523.

**SELLE, P.H. and RAVINDRAN, V.** (2007) Microbial phytase in poultry nutrition. *Animal Feed Science and Technology* **135**: 1-41.

**WALDROUP, P.W., KERSEY, J.H., SALEH, E.A., FRITTS, C.A., YAN, F., STILBORN, H.L., CRUM, R.C. and RABOY, V.** (2000) Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. *Poultry Science* **79**: 1451-1459.

**YAN, F., KERSEY, J.H., FRITTS, C.A. and WALDROUP, P.W.** (2003) Phosphorus requirements of broiler chicks six to nine weeks of age as influenced by phytase supplementation. *Poultry Science* **82**: 294-300.

**YOSHIDA, M. and HOSHII, H.** (1977) Improvement of biological assay to determine available phosphorus with growing chicks. *Japanese Poultry Science* **14**: 33-43.