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**Justifying Phytogenic Feed Additive Matrix Values in
Conjunction with Exogenous Feed Enzymes**

Laurel K. Shires

**Thesis submitted to the
Davis College of Agriculture, Natural Resources and Design
at West Virginia University
in partial fulfillment of the requirements for the degree of**

**Master of Science
in
Animal and Nutritional Sciences**

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**Morgantown, West Virginia
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ABSTRACT

Justifying Phytogenic Feed Additive Matrix Values in Conjunction
with Exogenous Feed Enzymes

Laurel K. Shires

Phytogenic feed additives are purported to possess antimicrobial properties as well as nutrient sparing characteristics that may aid in alleviating high diet costs; however, in order for PFA's to assist nutritionists in decreasing diet cost, matrix values must be determined and implemented in feed formulation. *Study 1* evaluated proposed matrix values for a commercially available PFA and assessed nutrient sparing when the product was combined with commercial phytase, carbohydrase and protease. The most remarkable proposed matrix values were 32.2 kcal/kg for metabolizable energy and 0.07% for both Ca and AP. The objective of *Study 2* was to determine true amino acid digestibility **(TAAD)** and nitrogen corrected true metabolizable energy **(TME$_n$)**. Dietary treatments for both studies included a basal, basal with phytogenic product matrix value, basal with phytogenic product matrix value and phytogenic product, and similar treatments evaluating the phytogenic product matrix with exogenous enzyme products. Decreasing the basal diet by the proposed phytogenic matrix values decreased broiler live weight gain and increased feed conversion ratio (P≤0.05). However, when the same diet included the phytogenic feed additive, live weight gain and feed conversion ratio were restored to that of the basal diet (P>0.05). The proposed matrix values of the specific PFA tested were justified. However, the PFA was not additive or synergistic with exogenous enzymes. Nitrogen corrected true metabolizable energy and TAAD data did not differ when the diets varied based on the PFA per se (P>0.05). However, when the PFA was incorporated using proposed matrix values and used in conjunction with exogenous enzymes and their matrix values, TME$_n$ and several tested TAAD values were decreased (P≤0.05). Mechanistic speculation for decreased nutrient digestibility may perhaps involve reductions in gut microflora due to the PFA as well as simultaneous reduction in substrate concentrations.

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TABLE OF CONTENTS PAGE

LIST OF TABLES

CHAPTER TWO

LITERATURE REVIEW

I. PHYTOGENIC FEED ADDITIVES

Recently, consumers have expressed growing concerns surrounding the possible development of antibiotic resistant bacteria resulting from excessive, low level use in livestock production. This has prompted the ban of numerous antibiotics in animal feed throughout the world. The United Kingdom banned the use of penicillin and tetracycline for growth promotion in the 1970s. The United States banned the use of enrofloxacin in 2005. The European Union enforced a partial ban on antibiotics in 1999 and a complete ban was issued in 2006 [1]. The Council for Agricultural Science and Technology estimates that a ban on low level antibiotics in all livestock feed would result in collective losses of $1 million to $28 billion over a five year period. In addition, broiler producers could lose up to $12 billion in the same amount of time if no substitutes are used in place of sub-therapeutic antibiotics [2, 3]. The ever changing nutritional concerns of society have spiked interest in the production and consumption of natural foods; phytogenic feed additives, along with organic acids and probiotics, may perhaps be viable alternatives to traditional antibiotic and growth promoter use and in 1984 companies took the first steps toward developing such products [4].

The prefix 'phyto' refers to plants hence; phytogenic feed additives (often called phytobiotics or botanicals) and are derived from certain plant parts such as: stems, leaves, roots, and/or flowers and contain a combination of essential oils, pungent substances, bitter substances, saponins, tannins, and flavanoids [5].   Phytogenic feed additives are primarily composed of essential oils which are concentrated, hydrophobic liquids containing highly volatile substances isolated by a physical process such as cold expression, steam distillation or fermentation, from an

1

odoriferous plant of a single botanical species. The oil bears the name of the plant from which it is derived…Such oils were called essential because they were thought to represent the very essence of odor and flavor [6].

Phytogenic feed additives are purported to promote natural digestion while improving performance along with other various modes of action such as decreasing bacterial colony counts and fermentation products (ammonia and biogenic amines), reducing activity of the gut associated lymphatic system, increasing prececal nutrient digestion, and possessing antioxidative properties [7]. Essential oils possess strong aromatic properties which increase organo-leptic stimulation while bitter substances, stemming from herbs, are reported to regulate appetite and stimulate the secretion of gastric juices. Pungent substances such as paprika, garlic and onion are purported to function by increasing blood circulation leading to faster detoxification of the whole metabolism. All of these stimulate the secretion of digestive enzymes of mucosa and pancreas which increase nutrient digestibility [7].

Over the past decade, there has been a significant increase in scientific evidence surrounding the use of phytogenic feed additives and the potential for these additives to exhibit positive effects for animal production. However, it should be noted that the use of phytogenic feed additives is surrounded by conflicting research reports. While essential oils are said to increase organo-leptic or sensory stiumulation, there are few choice feeding studies available for conclusive evidence. Jugl-Chizzola *et al*. showed dose related depressions of palatability in pigs fed essential oils from fennel and caraway [8]. A feeding trial conducted by Muhl and Leibert found that swine fed diets containing an essential oil blend of thymol and carvacrol did not exhibit effects of improved digestion[11]. While on the contrary, there are reports of improved

feed utilization involving swine fed diets containing phytogenic feed additives. Kroismayr *et al*. reported that performance variables were increased when piglets were fed a mix of essential oils stemming from oregano, anis, and citrus peels [12]. Increased feed intake is often seen in swine consuming feed supplemented with essential oils; however, this increase in feed intake may be a common result of other growth promoting feed additives such as organic acids, antibiotics and probiotics or a reflection of increased consumption capacity of swine and not directly related to the phytogenic feed additive per se [13]. Limited research exists surrounding the growth promoting effects of phytogenic feed additives in poultry however, Buchanan *et al*. reported that broilers fed diets containing plant extract blends (microencapsulated essential oils, bitter and pungent substances) had lower feed conversion ratios, improved live weight gain, and higher breast yield [3].   Windisch *et al*. completed an inclusive review regarding phytogenic feed additives and the proposed modes of actions of these products . The general conclusion encompassed in this review supports the idea that phytogenic feed additives may add to the set of non antibiotic growth promoters thereby increasing animal performance naturally [5].

Advantages of Phytogenic Feed Additive Application:

- Improved performance (Live weight gain and feed conversion ratio)
- Decreasing harmful bacteria in the gastrointestinal tract
- Increased feed intake (swine)
- Reduced mortality
- Increased egg production
- Improved barn climate (ammonia and biogenic amine reduction)
- Antioxidative actions

II. Antimicrobial Action and Improving Gut Function

One of the main concerns involving poultry production, or any livestock production for human food, is the spread of disease from animal to man, better known as zoonotic diseases [14]. For this reason, there have been numerous studies involving phytogenic feed additives aiming at reducing harmful bacteria colonies in poultry. The antimicrobial effects are purported to arise from the hydrophobic essential oils ability to intrude into the bacterial cell membranes, disintegrate the structures which results in ion leakage [15]. Salmonella infections are asymptomatic in poultry flocks, but are associated with widespread human illness throughout the world. These infections can stem from raw or undercooked eggs as well as broiler meat. Salmonella is known to colonize in the gastrointestinal tract of poultry which has led to the development of measures to increase colonization resistance by manipulating the make-up of intestinal microbiota [16,17]. This is known as the competitive exclusion principle (CE), which states that "…two species competing for the same resources cannot stably coexist, if the ecological factors are constant. Either of the two competitors will always take over the other which leads to either the extinction of one of the competitors or its evolutionary or behavioral shift towards a different ecological niche" [17]. McElroy et al. found that both low and high levels of capsaicin, an essential oil from chili peppers, administered to birds 21, 28, and 42 days of age increased resistance to Salmonella without affecting weight gain, feed consumption, or carcass quality characteristics [18]. Lis-Balchin et al. studied the bioactivity of selected plant essential oils in vitro against *Listeria monocytogenes.* *L. monocytogenes* is present in soil, water, vegetables, and intestinal contents of a variety of birds, fish, insects and other animals.

Human listeriosis is an erratic disease, which is associated with consumption of contaminated milk, soft cheese, under-cooked meat, unwashed raw vegetables, and cabbage [19].

 93 essential oils were studied against 20 different strains of *L. monocytogenes*; white camphor, lemon verbena, angelica root, cassia, cinnamon leaf, clove leaf, basil, bergamot, pimento, bay, *Eucalyptus radiata* and *citriodora*, tea-tree and lemongrass were effective against all 20 strains [19].

Coccidiosis significantly affects the poultry industry in terms of economic loss which has been estimated at $1-3 billion dollars annually [20]. Coccidiosis results from the species *Eimeria* which lives and multiplies in the intestinal tract resulting in tissue damage which can decrease digestion and nutrient absorption. The tissue damage resulting from coccidiosis may also increase the risk for exposure to other types of pathogens such as *Clostridium* or *Salmonella* [21].

Decreasing microbial activity is of high importance due to the effects associated with the disruption of enteric balance resulting from increased numbers of harmful bacteria. Bacterial by products are known to disturb pH balance in the GIT thereby reducing endogenous enzyme efficacy. The production of biogenic amines is also regarded as a serious problem mainly because their production results from the decarboxylation of essential amino acids. Therefore, the reduction of harmful bacterial colony counts may increase the supply of essential amino acids [22].

III. Antioxidative Properties

Many phytogenic compounds possess antioxidative properties which may help reduce oxidation of feed lipids similar to antioxidants that are currently employed in animal feeds ie; α-tocopheryl acetate and BHT. Trials detailing information surrounding the application of traditional antioxidants versus the use of phytogenic feed additives as antioxidants are rapidly increasing [23,24]. During the past decade, phytogenic feed additive compounds have clearly demonstrated antioxidative effects in both meat and eggs [23,24,25,26] Essential oils (primarily from the Labiatae family) have been used in both human and companion animal food as natural antioxidants for quite some time [27] Govaris *et al*. detailed the effects of dietary application of antioxidants versus post mortem application to carcass meat and concluded that antioxidative effects are more pronounced when added to the diet [28]. Dietary additions of oregano essential oil improved tissue retention of α-tocopheryl and a combination of oregano and rosemary oil further demonstrated these effects [23,29]

PFA Components and Proposed Modes of Action	
Component	Mode of Action
Essential Oil	Increase digestive secretions & N retention, appetizing
Bitter Substances	Increase digestive secretions & nutrient retention, appetizing
Pungent Substances	Increase circulation & metabolic processes, detoxification
Saponins	Enhance permeability of the gut wall, increase nutrient adsorption, reduce ammonia
Acid Complex	Acts against pathogenic bacteria, improved health status

IV. Exogenous Feed Enzymes

The US animal feed industry currently faces tremendous hurdles due to increased ingredient cost and nutrient inconsistencies among source. Any solution to these problems must make sense economically and not comprise performance or health of the animal. Non-ruminant commercial diets typically consist of two or three ingredients that account for >75% of the diet [30]. These ingredients per se contain various antinutritional factors (ANF); however, their increased cost has dictated the use of alternative ingredients containing sometimes greater quantities of ANF. It is under these conditions that exogenous enzyme supplementation that enables improved nutrient availability of feed ingredients containing ANF would be beneficial to both animal and producer. However, how nutritionists effectively employ exogenous feed enzymes in feed formulations is another hurdle entirely.

Phytase and non starch polysaccharide degrading enzymes are two exogenous feed enzymes that present problems for nutritionists that are attempting to obtain maximum enzyme value. The main purpose of phytase is to increase availability of plant phytate phosphorus but other minerals, carbohydrates and amino acids can be bound to phytic acid and made available due to phytate phosphorus digestion [31]. Non-starch polysaccharide degrading enzymes are used to increase digestibility of non-starch carbohydrates in the diet and reduce viscosity in the digesta that consequently improves overall nutrient digestibility [32]. Enzyme value is based on the quantities of specific nutrients expected to be spared by exogenous enzyme inclusion.

Simons et al. found when microbial phytase was added at a concentration of 1500 FYT/ kg feed to low-phosphorus broiler diets, the availability of P increased 60% while the P in excreta decreased 50% [33]. Baker et al. showed an increase in both mineral digestibility and

7

apparent metabolizable energy (AME) using various phytase concentrations. Ca digestibility

increased when two phytase products (1 and 2) were added at concentrations of 500 FYT/kg and

250 FYT/kg, respectively. AME increased in all treatments containing phytase [34]

Choct et al. found that diets containing low AME wheat supplemented with glycanase

significantly increased the AME of the deficient wheat from 12.02 MJ/kg of dry matter to 14.94

MJ/kg of dry matter. The low AME wheat + enzyme treatment also significantly decreased

digesta viscosity [32].   Santos et al. showed that supplementation of xylanase in wheat based

diets significantly decreased digestive viscosity therefore increasing nutrient digestibility in toms

[35]. While research has proven the application of these enzymes can save significant dollars in

the industry, nutritionists are still forced to gamble whether to utilize extra substrate nutrient

matrix values fully. If the extra substrate nutrient values are not obtained after the matrix values

are applied, the results could be very costly.

V. Matrix Values

Matrix values for various products are determined through extensive in-house analyses

and implemented to estimate the nutrient sparing effects for metabolizable energy, amino acids,

minerals, etc. For instance, proposed matrix values for a commercially available phytogenic feed

additive are 14.6 kcal/lb for metabolizable energy, .03% for lysine, .02% for both methionine

and threonine, and .07% for calcium and available phosphorus. With these values, it would be

possible for nutritionists to formulate nutritionally adequate diets while decreasing, by the

respective amounts, the aforementioned nutrients. As ingredient cost continues to rise, correct

application of feed additive matrix values is crucial in relieving high cost. Traditionally,

8

exogenous enzymes have been employed to alleviate high diet costs. However, the potential

may exist for simultaneous phytogenic feed additive and exogenous enzyme application into

livestock feed to further decrease ingredient and overall diet cost.

VI. Determining TME_n and TAAD Values

Metabolizable energy (ME) is the measure of the energy available to the birds via the diet [36].

ME can be expressed as apparent (AME) or true (TME) metabolizable energy [37]. AME was

the most common measure of ME in the past, however this measurement does not separate total

excretory energy into independent estimates of fecal plus endogenous urinary energy [38]. In

contrast, TME recognizes fecal and urinary energy (from non-dietary sources) as metabolized

energy and is considered a more direct measure of energy availabilty [37,39]. AME values are

derived from data obtained from test birds that are confined to metabolism cages in which

experimental diets are fed ad libitum and total feed and excretory output are measured. This is

known as the total collection method [40]. Bomb calorimetry is then employed to determine

gross energy of both feed and exreta. Both AME and TME must be corrected to zero nitrogen

balance so the values derived using birds with different nitrogen requirements (growing v.

mature) will be comparable. 8.22 kcal (amount of energy obtained when uric acid is completely

oxidized) are added or subtracted from the ME value for each gram of nitrogen lost or gained to

account for energy required in the excretion of urinary energy [37]. AME_n is derived from the

following equation:

A= total feed energy

B= total excreta energy

C= total excreta energy of fasted roosters

D= N correction factor (8.22 kcal/g).

AME_n (kcal/g)= (A-B-D)/ amount of feed (g)

Sibbald described the direct method for determining TME in 1976. Two groups of birds, control

and experimental, are fasted for twenty-four hours, and the experimental birds are then precision

fed a known quantity of feed (20-30g). The birds are then placed in raised wire cages and

excreta are collected for 48 hours. Each bird may serve as its own negative control in which

fecal and urinary energy estimates are derived [41]. Control birds are fasted for 24 hours and

excreta is then collected for 48 hours. TME_n is derived from the following equation:

A= total feed energy

B= total excreta energy

C= total excreta energy of fasted roosters

D= N correction factor

TME_n (kcal/g) = (A-B+C-D)/ amount of feed (g)

TME_n values are more commonly utilized than those of AME_n. This is due to fundamental

improvements upon the AME_n method such as: providing nutrient values more similar to the

actual biological value for poultry than previously achieved, decreased completion time, and

easily reproducible methods [42].

Several modified methods exist for the determination of TME_n and TAAD. TAAD values may be derived from methods similar to those that determine TME_n. The most commonly utilized method is that of McNab and Blair in which adult, male Single Comb White Leghorn roosters are cecectomized to determine energy and amino acid digestibilities of feed ingredients or complete diets [43]. The concentration of amino acids in the excreta is subtracted from the concentration of amino acids in the feedstuffs and related back to the amount of amino acid intake. Endogenous amino acid losses are determined through the use of starved birds or birds fed a protein free diet such as cornstarch [44].

The ceca comprise a major part of the large intestine in poultry and provide an environment suitable for numerous microorganisms [45]. The benefits of hindgut fermentation to poultry are unclear and past research has demonstrated that amino acids are not absorbed in the hindgut in significant quantities [46]. Researchers have stated that cecectomized birds should be used to prevent overestimation of amino acid digestibility in feedstuffs [47,48]

VII.   Future Research

Further justification of the proposed matrix values for this product may be pertinent in explaining the results of the following experiment. Future research will involve the justification of both Ca and AP matrix values of the same commercially available phytogenic feed additive (Appendix I). Justification of these matrix values will be determined through live bird performance as well as bone mineralization assays similar to those described in Appendix II.

References

1. Food Marketing Institute. 2006. Low level use of antibiotics in livestock and poultry. http://www.fmi.org/media/bg/antibiotics.pdf
2. Council for Agricultural Science and Technology. 1981. Antibiotics in animal feeds. Report No. 88. Counc. Agric. Sci. Technol., Ames, IA.
3. Buchanan, N.P., J. Hott., S. Cutlip., A. Rack., A. Asamer., J. Moritz., 2008. The Effects of a Natural Antibiotic Alternative and a Natural Growth Promoter Feed Additive on Broiler Performance and Carcass Quality. *Journal of Applied Poultry Research*. 2008. 17:202-210.
4. Ek Sze, DVM. Delacon Biotechnik. Phytogenic Feed Additive: A Natural Performer. http://www.livestockasia.com/conference_paper/slide/sze.pdf.
5. Windisch, W., K. Schedle., C. Plitzner., A. Kroismayr., 2008.   Use of Phytogenic Products as Feed Additives for Swine and Poultry. *Journal of Animal Science* 2008. 86:140-148.
6. "essential oil." Encyclopedia Britannica. 2008. Encyclopedia Britannica Online. 01 Oct. 2008 <http://www.britannica.com/EBchecked/topic/193135/essential-oil>.
7. Delacon Phytogenic Feed Additives. 2004. Biostrong 505—The preventative solution for profitable poultry fattening. Delacon Biotechnik, Steyregg, Austria
8. Jugl-Chizzola, M., J. Spergser, F. Schilcher, J. Novak, A. Bucher, C. Gabler, W. Hagmuller, and K. Zitterl-Eglseer. 2005. Effects of *Thymus vulgaris* L. as feed additive in piglets and against haemolytic *E. coli* in vitro. Berl. Munch. Tierarztl. Wochenschr. 118:495–501.
9. Freitag, M., H. U. Hensche, H. Schulte-Sienbeck, and B. Reichelt. 1998. Biological effects of conventional and alternative performance enhancers.
10. "zoonotic disease." MedicineNet. 2004. 01 Oct. 2008. http://www.medterms.com/script/main/art.asp?articlekey=20220
11. Muhl, A., Leibert, F., 2007. No impact of a phytogenic feed additive on digestion and unspecific immune reaction in piglets. *Journal of Animal Physiology and Animal Nutrition*. 91:426-431.
12. Kroismayr, A., Schedle, K., Sehm, J., Pfaffl, MW., Plitzner, C., Foissy, H., Ettle, T., Mayer, H., Schreiner, M., Windisch, W. 2008. *Effects of antimicrobial feed additives on gut microbiology and blood parameters of weaned piglets.* Die Bodenkultur. 59:111-120.
13. Freitag, M., H. U. Hensche, H. Schulte-Sienbeck, and B. Reichelt. 1998. Biological effects of conventional and alternative performance enhancers.
14. "zoonotic disease." MedicineNet. 2004. 01 Oct. 2008.
15. Greathead, H., 2003. Plants and plant extracts for improving animal productivity. Proceedings of the Nutrition Society. 62:279-290.

16. Revelledo, L., A.J.P. Ferreira., G.C. Mead., 2006. Prospects in Salmonella
17. Control: Competitive Exclusion, Probiotics, and Enhancement of Avian Intestinal Immunity. *Journal of Applied Poultry Research*. 15: 341-351.

18. McElroy, Audrey., Hot Pepper Oil May Prevent Salmonella in Poultry., Science Daily on the Web. 20 Aug. 2001., 15 Sept. 2008., http://www.sciencedaily.com/releases/2001/08/010820071840.htm.

19. Lis- Balchin, M., S.G. Deans., 1996. Bioactivity of selected plant essential oils against *Listeria monocytogenes. Journal of Applied Microbiology*. 82: 759-762.

20. Williams, R.B., 1999. A compartmentalized model for the estimation of the cost of coccidiosis to the world's chicken production industry. International *Journal of Parasitology*. 29:1209-1229

21. Helm, J.D. 1999. Coccidiosis in Poultry. Livestock Poultry Health Programs. <http://www.clemson.edu/public/lph/ahp/disease_links/images/coccidia.pdf.>.

22. Roth, F.X., Kirchgessner, M. 1998. Organic acids as feed additives for young pigs: nutritional and gastrointestinal effects. *Journal of Animal and Feed Sciences*. 8:25-33.

23. Botsoglou, N.A.,Christaki, E., Florou-Paneri, P., Giannenas, I., Papageorgiou, G., Spais, A.B., 2004. The effect of a mixture of herbal essential oils or alpha tocopheryl acetate on performance parameters and oxidation of body lipids in broilers. *South African Journal of Animal Science.* 34:52-61.

24. Botsoglou, N.A., Florou-Paneri, P., Botsolgou, E., Dotas, V., Giannenas, I., Koidas A., Mitrakos, P., 2005. The effect of feeding rosemary, oregano, saffron and alpha-tocopheryl acetate on hen performance and oxidative stability of eggs. *South African Journal of Animal Science*. 35:143-151.

25. Janz, J.A.M., Morel, P.C.H., Wilkinson, B.H.P., Purchas, R.W., 2007. Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. *Meat Science* 75: 350-355.

26. Young, J.F., Stagsted, J., Jensen, S.K., Karlsson A.H., Henckel, P., 2003. Ascorbic acid, alpha tocohperol, and oregano supplements reduce stress induced deterioration of chicken meat quality. *Poultry Science*. 82: 1343-1351.

27. Cuppett, S.L., Hall, S.A., 1998. Antioxidant property of Labiatae. Advances in Food and Nutrition Research. 42: 245-271.

28. Govaris, A., Botsoglou, N., Botsoglou, E., Ambrosiadis, I., 2004. Dietary versus post mortem use of oregano and/or alpha tocopheryl acetate in turkeys to inhibit development of lipid oxidation in meat during refrigerated storage. *International Journal of Food Sciences and Nutrition*. 55:115-123.

29. Basmacioglu, H., Tokusoglu, O., Ergul M., 2004. The effect of oregano and rosemary essential oils or alpha tocopheryl acetate on performance and lipid oxidation of meat enriched with n-3 PUFA's in broilers. South African Journal of Animal Science.34:197-210.

30. Bedford, M.R., Schulze, H., 1999. Exogenous Enzymes for Pigs and Poultry. Nutrition Research Reviews 11:91-114.

31. Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition : their current value and future benefits. *Animal Feed Science and Technology* 86:1-13.

32. Choct, M., Hughes, R.J., Trimble, R.P., Angkanaporn, K., Annison, G. 1994. Non-Starch Polysaccharide-Degrading Enzymes Increase the Performance of Broiler Chickens Fed Wheat of Low Apparent Metabolizable Energy. *Journal of Nutrition* 1235 (3):485-492

33. Simons, P.C.M, Versteegh H.A.J,Jongbloed A.W., Kemme P.A.,Slump P., K.D. Bos, Wolters M.G.E., Beudeker R.F. ,and Verschoor G.J., 1990. Improvement of phosphorus availability by microbial phytase in broiler and pigs. *British. Journal of Nutrition*. 64: 525-541.

34. Baker, N.J., Parsons A.S., and Moritz, J.S., 2007. Effects of Various Phytase Concentrations in Diets with Low-phytate Corn on Broiler Chick Performance and Nutrient Use**.** *International Journal of Poultry Science* 6 (2): 77-84.

35. A.A. Santos Jr, P.R. Ferket, J.L. Grimes and F.W. Edens. 2004. Dietary Supplementation of Endoxylanases and Phospholipase for Turkeys Fed Wheat-based Rations. *International Journal of Poultry Science* 3 (1): 20-32.

36. Vorha, P., 1966. Energy concepts for poultry nutrition. World's *Poultry Science Journal*. 22:6-24.

37. Harris, L.E., 1966. Biological energy interrelationships and glossary of energy terms. National Academic Science Publication. 1411.

38. Sibbald, I.R., 1976. A bioassay for true metabolizable energy in feedingstuffs. *Poultry Science*. 55: 303-308.

39. Owen, R.B., Jr., Reinecke, J., 1979. Bioenergetics of breeding dabbling ducks. 71-93.

40. Vohra, P., 1972. Evaluation of metabolizable energy for poultry. *World's Poultry Science Journal*. 29:204-214.

41. Sibbald, I.R., Price, K., 1980. Variability in metabolic plus endogenous energy losses of adult cockerels and in the true metabolizable energy values and rates of passage of dehydrated alfalfa. *Poultry Science*. 59:1275-1279.

42. Dudley-Cash,W.A., 2009. A landmark contribution to poultry science- A bioassay for true metabolizable energy in feedingstuffs. *Poultry Science*. 88:832-834.

43. McNab, J.M., Blair, J.C., 1988. Modified assay for true and apparent metabolizable energy based on tube feeding. *British Poultry Science*. 29:697-707.

44. Sibbald, I.R., 1987. Estimation of bioavailable amino acids in feedingstuffs for poultry and pigs: a review with emphasis on balance experiments. Can. J. of An. Sci., 67:221-330.

45. Barnes, E.M., Mead, G.C., Barnum, D.A., Harry, E.G., 1972. The intestinal flora of the chicken in the period of 2 to 6 weeks of age, with particular reference to anaerobic bacteria*. British Poultry Science*. 13:311-326.

46. Webb, K.E., 1990. Intestinal absorption of protein hydrolysis products: A review. *Journal of Animal Science*. 68:3011-3022.

47. Johnson, R.J., 1992. Principles, problems, and application of amino acid digestibility in poultry. *World's Poultry Science Journal.* 48: 232-246.

48. Ragland, D., Thomas, C.R., Elkin, R.G., Shafer, D.J., and Adeola, O., 1999. The influence of cecectomy on metabolizable energy and amino acid digestibility of select feedstuffs for White Pekin ducks. *Poultry Science.* 78:707-713.

Justifying Phytogenic Feed Additive Matrix Values in Conjunction with Exogenous Feed Enzymes

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SUMMARY

Phytogenic feed additives are purported to possess antimicrobial properties as well as nutrient sparing characteristics that may aid in alleviating high diet costs; however, in order for PFA's to assist nutritionists in decreasing diet cost, matrix values must be determined and implemented in feed formulation. *Study 1* evaluated proposed matrix values for a commercially available PFA and assessed nutrient sparing when the product was combined with commercial phytase, carbohydrase and protease. The most remarkable proposed matrix values were 32.2 kcal/kg for metabolizable energy and 0.07% for both Ca and AP. The objective of *Study 2* was to determine true amino acid digestibility **(TAAD)** and nitrogen corrected true metabolizable energy **(TME$_n$)**. Dietary treatments for both studies included a basal, basal with phytogenic product matrix value, basal with phytogenic product matrix value and phytogenic product, and similar treatments evaluating the phytogenic product matrix with exogenous enzyme products. Decreasing the basal diet by the proposed phytogenic matrix values decreased broiler live weight gain and increased feed conversion ratio (P≤0.05). However, when the same diet included the phytogenic feed additive, live weight gain and feed conversion ratio were restored to that of the basal diet (P>0.05). The proposed matrix values of the specific PFA tested were justified. However, the PFA was not additive or synergistic with exogenous enzymes. Nitrogen corrected

true metabolizable energy and TAAD data did not differ when the diets varied based on the PFA

per se (P>0.05). However, when the PFA was incorporated using proposed matrix values and

used in conjunction with exogenous enzymes and their matrix values, TME_n and several tested

TAAD values were decreased (P≤0.05). Mechanistic speculation for decreased nutrient

digestibility may perhaps involve reductions in gut microflora due to the PFA as well as

simultaneous reduction in substrate concentrations.

Key words: phytogenic additives, matrix values, exogenous enzymes, true amino acid

digestibility

DESCRIPTION OF PROBLEM

Consumers have increasingly expressed concern about the development of antibiotic

resistant bacteria resulting from continual, low level incorporation of antibiotics in livestock

feed. This has prompted the ban of numerous antibiotics in animal feed throughout the world.

Phytogenic feed additives along with organic acids and probiotics, represent alternatives to

traditional antibiotic use and in 1984 companies took the first steps toward developing such

products [1]. The prefix 'phyto' refers to plants; hence, phytogenic feed additives (often called

phytobiotics or botanicals) are derived from certain plant parts such as: stems, leaves, roots,

and/or flowers and contain a combination of essential oils, pungent substances, bitter substances,

saponins, tannins, and flavanoids [2]. Phytogenic feed additives typically contain a high

percentage of essential oils which are concentrated, hydrophobic liquids containing highly

volatile substances isolated by a physical process such as cold expression, steam distillation or

fermentation, from an odoriferous plant of a single botanical species. The oil bears the name of

16

the plant from which it was derived. Such oils were called essential because they were thought

to represent the very essence of odor and flavor [3]. Phytogenic feed additives are marketed as

promoting natural digestion and improving performance associated with modes of action such as

decreasing harmful bacterial colony counts and fermentation products (ammonia and biogenic

amines), reducing activity of the gut associated lymphatic system, increasing prececal nutrient

digestion, and having antioxidative actions [4].   Essential oils possess strong aromatic properties

which increase organoleptic stimulation while bitter substances, stemming from herbs, are

reported to regulate appetite and stimulate the secretion of gastric juices. Pungent substances

such as paprika, garlic and onion are purported to function by increasing blood circulation,

leading to faster detoxification of the metabolism. All of these stimulate the secretion of

digestive enzymes of mucosa and pancreas which increase nutrient digestibility [5]. Perhaps

when these products are used alongside conventional exogenous feed enzymes, feed digestion,

and consequent animal performance could be further enhanced. The objective of *Study 1* was to

evaluate the proposed matrix values for a commercially available PFA and to assess nutrient

sparing when the product was combined with a commercial phytase, carbohydrase, and protease.

Assessment was based on growth performance of broiler chickens. The objective of *Study 2* was

to evaluate the proposed matrix values for a commercially available PFA through TAAD and

TME_n measures using cecectomized Single Comb White Leghorn rooster models.

MATERIALS AND METHODS

   Feed Manufacture. Eight dietary treatments, formulated to meet Cobb-Vantress Inc. [6] least

cost starter recommendations, were manufactured at West Virginia University's Pilot feedmill

(Table 1). Each of the eight dietary treatments totaled 363 kg and were subsequently divided

into four 91 kg batches. The decreased batch size was created in order to increase mixer

uniformity based on WVU feed mill capabilities. Each batch was mixed in a horizontal ribbon

mixer for twenty minutes[7].

Study 1. A total of 1,412 male Cobb 500 broilers were obtained from a commercial hatchery at

hatch and 1,344 of these broilers were selected based on weight to create uniformity in initial pen

weight. Weighing occurred on day four and groups of selected birds were randomly allotted to 1

of 64 floor pens [0.69 × 2.44 m (2.26× 8.00 ft)] located in 2 rooms joined by a woven wire

barrier that allowed heat and ventilation to move freely between the rooms. Rooms were located

in a cross-ventilated negative-pressure house with forced-air brooders. Each room was

considered one block, and each block was composed of a group of 32 pens. Broilers were placed

at a stocking density of 21 birds/pen [0.065 m^2/bird (0.70 ft^2/bird)] and were provided with feed

and water, supplied through Kuhl feed pans adapted to hoppers and Ziggity nipple drinkers, for

ad libitum consumption[8,9]. During the 1-3 d pretest period, broiler chicks were fed a nutrient

deficient diet that utilized the PFA matrix without the PFA. This was done so birds would not be

on a high plane of nutrition that could confound treatment effects.   On day four, each of the

eight experimental diets were randomly assigned to pens within each block. The matrix values

established for this particular PFA were 32.2 kcal/kg (14.6 kcal/lb) for metabolizable energy,

0.03% for Threonine, 0.02% for both Methionine and Lysine, and 0.07% for both AP and

calcium [4]. The matrix values for the carbohydrase-protease blend were 75.8 kcal/kg (34.4

kcal/lb) for metabolizable energy and 0.5% for crude protein [10]. The phytase that was

implemented had calculated matrix values of 0.1% for available phosphorus [11]. Temperature

of the rooms was initially maintained at 32.2°C (90.0°F) and incrementally decreased to 23.9°C

(75.0°F) during the experimental period of 4 to 21 days. Live weight gain **(LWG)**, feed intake

(FI), and feed conversion ratio **(FCR)** were determined from 4-21 days. Feed conversion ratio

calculations included mortality weight. All animals were reared according to protocols

established by the West Virginia University Animal Care and Use Committee [ACUC # 08-

0894].

Study 2. A total of 32 cecectomized SCWL roosters, approximately 50 weeks of age, were used

to estimate TAAD as well as TME_n for the eight treatments used in the previous study using a

modified procedure of Sibbald [12]. On day one of the experiment, roosters were placed on a

three week pre-test utilizing the specific diet to be precision fed. Feed and water were supplied

ad libitum during this phase. Upon completion of the pre-test, roosters were placed in

environmentally controlled rooms containing raised wire cages. The room consisted of four

blocks based on location, and each block was composed of eight cages. Diets were randomly

assigned to caged birds within each block. Feed was withheld for 24 hours to ensure proper

emptying of the gastrointestinal tract; birds were then precision fed 30 grams of feed from one of

the eight dietary treatments corresponding to the pre-test diets. Total excreta were collected

over a 48 hour period. Upon completion, roosters were placed back on their respective pre-test

diets for three weeks in order to provide adequate rest and recovery. Rooster models were then

used in a second series of this study to obtain eight replications for each dietary treatment. Each

bird was precision fed cornstarch for amino acid quantification and feed was withdrawn for 24

19

hours and excreta collected for 48 hours in order to quantify endogenous energy losses. After

the experiment, each bird was used as its own control, similar to Latshaw and Freeland [13]. ,

Collected excreta were lyophilized, weighed, and ground. Feed and excreta samples were

submitted to commercial laboratories to quantify amino acids, gross energy, and nitrogen

[14],[15],[16],[17].

Statistical Analysis

Analyses were completed using GLM ANOVA procedure from the SAS Institute [18] and when

significant differences were detected, treatment means were separated and compared using

Fisher's LSD. Means were considered significantly different at ($P \leq 0.05$). Pre-planned

orthogonal contrasts of treatments three vs. four and treatments seven vs. eight were employed to

determine the effects of the PFA on metabolism of reduced nutrient diets. Although our omnibus

F-test was non significant, several of our contrasts demonstrated significant treatment differences

(Table. 3). This situation has been detailed by Milliken and Johnson [19].

RESULTS and DISCUSSION

  Study 1. Performance data for the four to twenty-one day period are illustrated in Table

2. The basal diet top dressed with the PFA (Treatment 2) demonstrated similar LWG values and

increased FCR values when compared to the basal diet (Treatment 1) ($P>0.05$) possibly resulting

from the increase in FI due to the PFA. When the PFA matrix values were applied to dietary

treatments lacking the PFA (Treatment 3), broiler LWG was decreased and feed conversion ratio

was increased compared to broilers fed the basal diet (Treatment 1) ($P \leq 0.05$) . However, when

the PFA matrix was applied and the PFA was included (Treatment 4), LWG and FCR were restored to values comparable to that of the basal diet (Treatment 1) ($P>0.05$). The dietary treatment with enzyme matrices applied with enzymes (Treatment 5) demonstrated LWG and FCR values similar to that of the basal diet (Treatment 1) ($P>0.05$) demonstrating the efficacy of the exogenous enzymes used. The application of all matrices (exogenous enzyme matrices and PFA matrices) without the inclusion of PFA but with enzymes (Treatment 7), proved detrimental to both LWG and FCR as compared to the basal treatment (Treatment 1) ($P<0.05$). The inclusion of the PFA to this treatment (Treatment 8) could not restore LWG and FCR values. In fact, the combination of all products and their respective matrices proved sub-additive. This result could possibly be explained by full utilization of the PFA and enzyme matrix values resulting in a plane of nutrition too low to be overcome. Competition for similar substrates between the PFA, exogenous, and endogenous enzymes may also explain this phenomenon [20]. Potential increases in endogenous enzyme secretions due to the PFA may have been ineffective due to substrates being digested by exogenous enzymes resulting in an energy expensive metabolic state. In 2005, the European Food Safety Authority (EFSA) presented the opinion of the FEEDAP Scientific Panel on the safety and efficacy of a specific feed additive. It was concluded that negative interactions between feed additives and exogenous enzymes should not be dismissed [21].

Study 2. Energy and amino acid digestibility data from cecectomized SCWL roosters are illustrated in Table 3. No significant difference among TME_n or TAAD were demonstrated according to the omnibus F-test. However, pre-planned orthogonal contrasts were implemented

for treatment three vs. four and treatment seven vs. eight. A numerical increase in TME_n values were observed due to the incorporation of the PFA when comparing treatment two vs. one, four vs. three, and six vs. five (P>0.05). However, TME_n values had a tendency to decrease when all products matrices were applied, exogenous enzymes included, and the PFA included compared to the PFA being excluded (Treatment 7 vs. Treatment 8) (P<0.06). When comparing TAAD of treatments eight vs. seven, Aspartic acid, Glutamic acid, Valine, and Leucine, demonstrated trends toward decreased digestibility (P = 0.0798), (P = 0.0645), (P = 0.0510), (P = 0.0792), respectively ; while Proline, Alanine, Methionine, and Isoleucine showed decreased digestibility (P = 0.0117), (P = 0.0340), (P = 0.0230), (P = 0.0400), respectively. Similar speculations as described in *Study 1* results may also be relevant in explaining this data. There was no significant improvement in amino acid digestibility when comparing treatments three vs. four (P>0.05); however, proposed PFA matrix values for amino acids were relatively low to begin with.

CONCLUSIONS and APPLICATIONS

1. The PFA matrix values were justified when the product was used alone in the 4-21 d broiler performance study.

2. The PFA and exogenous enzymes with all matrices applied were sub-additive, increasing FCR and decreasing LWG in the 4-21 d broiler performance study and decreasing TME_n and several tested TAAD in the precision fed rooster study.

References and Notes

1. Ek Sze, DVM. Delacon Biotechnik. 2006.Phytogenic Feed Additive: A Natural Performer. 01. Oct. 2008 <http://www.livestockasia.com/conference_paper/slide/sze.pdf>.
2. Windisch, W., K. Schedle., C. Plitzner., A. Kroismayr., 2008. Use of Phytogenic Products as Feed Additives for Swine and Poultry. Journal of Animal Science 2008. 86:140-148.
3. Lee,KW, H. Everts , H. Kappert,M. Frehner, R. Losa ,A. Beynen.,2008. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. British Poultry Science Jul. 44(3): 450-7. 2003.
4. Delacon Phytogenic Feed Additives. 2004. Biostrong 510—The performizer for improved nutrient retention. Delacon Biotechnik, Steyregg, Austria.
5. Buchanan, N.P., J. Hott., S. Cutlip., A. Rack., A. Asamer., J. Moritz., 2008. The Effects of a Natural Antibiotic Alternative and a Natural Growth Promoter Feed Additive on Broiler Performance and Carcass Quality. Journal of Applied Poultry Research. 2008. 17:202-210.
6. Cobb-Vantress Inc., Siloam Springs, AR.
7. Davis Precision Mixer, Model S-1; H.C. Sons Manufacturing Co., Inc., Bonner Springs, KS.
8. Feed pan and hopper; Kuhl Corporation, Flemington, NJ.
9. Water system; Ziggity Systems Inc., Middlebury, IN.
10. Avizyme 1502; Danisco A/S, Cedar Rapids, IA
11. OptiPhos 2000; OptiPhos, Sheridan, IN
12. Sibbald, I. R. 1976. A bioassay for true metabolizable energy in feedingstuffs. Poult. Sci. 55:303–308. (Feed was withheld for 24 hours, approximately 30 grams of feed were precision fed, and excreta was collected for 48 hours.)
13. Latshaw, J.D., K. Freeland., 2009. Metabolizable Energy Values Determined with Intact and Cecectomized Roosters. Poult. Sci. 2008. 87:101-103.
14. CEPS, Central Analytical Laboratory, University of Arkansas, Fayetteville, AR
15. Experiment Station Chemical Laboratories, Agricultural Experiment Station, Univ. Missouri, Columbia, MO
16. Isoperibol oxygen bomb calorimeter model 1266, Parr Instrument Co., Moline, IL.
17. Kjeltec Auto 1030 analyzer, Foss Tecator, Eden Praire, MN.
18. SAS Institute. 2000. The SAS System for Windows 2000. Release 8.1. SAS Inst. Inc., Cary, NC.
19. Milliken, G. A., and D.E.,Johnson. (1992) Analysis of Messy Data: Designed Experiments. Van Nostrand Reinhold, New York.

20. Olokusi, O.A., A.J., Cowieson, O. Adeola., 2009. Age-Related Influence of a Cocktail of Xylanase, Amylase, and Protease or Phytase Individually or in Combination in Broilers. Poult Sci 2007. 86:77-86.
21. Anadon, A., M. Abroix Arzo, G. Bories, P. Brantom, J. Brufau de Barbera, A. Chesson, P. S. Concconcelli, J. de Knecht, N. Dierick, G. Flachowsky, A. Franklin, J. Gropp, A. K. Haldorsen, I. Halle, A. Mantovani, K. Peltonen, G. Rychen, P. Sanders, A. Soares, P. Wester, and W. Windisch. 2005. Opinion of the FEEDAP Panel on the safety and efficacy of the product Farmatan for rabbits and piglets. The EFSA Journal 222:1–20.

Table 1. Diet Formulations for Treatments 1-8 *(Study 1 and 2)*

Treatment[1]								
Ingredient	1	2	3	4	5	6	7	8
Corn	56.70	56.70	56.49	56.49	55.1	55.1	54.8	54.8
Soybean meal (48%)	24.54	24.54	23.51	23.51	23.63	23.63	22.73	22.73
Cellulose[2]	1.91	1.91	3.40	3.40	4.46	4.46	5.85	5.85
Wheat middlings	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
DDGS[3]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Tricalcium phosphate	1.50	1.50	1.14	1.14	.96	.96	0.60	0.60
Limestone	0.66	0.66	0.74	0.74	1.05	1.05	1.14	1.14
Porcine MBM[4]	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51
DL – methionine	0.29	0.29	0.29	0.29	0.30	0.30	0.29	0.29
NB 3000[5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.33	0.33	0.33	0.33	0.35	0.35	0.35	0.35
Salt	0.15	0.15	0.20	0.20	0.22	0.22	0.26	0.26
Threonine	0.14	0.14	0.14	0.14	0.15	0.15	0.14	0.14
PFA or sand	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Avizyme 1502[6]	--	--	--	--	0.05	0.05	0.05	0.05
Optiphos 2000[7]	--	--	--	--	0.0125	0.0125	0.0125	0.0125
Calculated Nutrient Values								
ME (kcal/kg)	3066.63	3066.63	3034.44	3034.44	2990.79	2990.79	2958.60	2958.60
CP (%)	19.55	19.55	19.03	19.03	19.00	19.00	18.53	18.53
Lysine (%)	1.19	1.19	1.16	1.16	1.18	1.18	1.15	1.15
Met+Cys (%)	0.88	0.88	0.86	0.86	0.87	0.87	0.85	0.85
Threonine (%)	0.76	0.76	0.74	0.74	0.75	0.75	0.73	0.73
Tryptophan (%)	0.24	0.24	0.23	0.23	0.23	0.23	0.23	0.23
Arginine (%)	1.24	1.24	1.20	1.20	1.20	1.20	1.16	1.16
Isoleucine (%)	0.84	0.84	0.82	0.82	0.81	0.81	0.79	0.79
Calcium (%)	0.84	0.84	0.77	0.77	0.84	0.84	0.77	0.77
Available P (%)	0.42	0.42	0.35	0.35	0.32	0.32	0.25	0.25
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[1] Treatments:
1=Basal
2=Basal + PFA
3=Basal+PFA matrix, no PFA
4=Basal+PFA & PFA matrix

5=Basal+enzymes, enzyme matrices, no PFA
6=Basal+enzymes, enzyme matrics, PFA
7=Basal+enzymes, enzyme matrices, PFA   matrix, no PFA
8= Basal+enzymes, enzyme matrices, PFA matrix, and PFA

[2] Solka-Floc; International Fiber Corporation, North Tonawanda, NY.

[3] Dried distillers grains with corn solubles
[4] Porcine meat and bone meal (55%)
[5] Supplied (per kg of diet): manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B6, 1.38 mg; niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; vitamin B12, 0.01 mg; vitamin E, 16.53 IU; vitamin D3, 2,133 ICU; vitamin A, 7,716 IU.
[6] Danisco USA Inc. Cedar Rapids, Iowa
[7] OptiPhos. Sheridan, IN

Table 2. Performance data for 4-21-d broiler chickens *(Study 1)*

Treatment[1]	Pen Feed Intake (kg)	Live Weight Gain (kg/bird)	Feed Conversion Ratio (kg/kg)	Mortality
1	20.66	.60 [a]	1.66 [c]	1.79
2	21.47	.60 [ab]	1.73 [ab]	1.19
3	20.61	.58 [c]	1.72 [b]	1.79
4	20.98	.60 [ab]	1.68 [bc]	0.60
5	20.93	.59 [abc]	1.69 [bc]	0.00
6	20.86	.59 [bc]	1.71 [bc]	1.19
7	20.81	.56 [d]	1.78 [a]	0.60
8	20.63	.55 [d]	1.78 [a]	0.00
ANOVA P values	0.0621	<0.0001	0.0002	0.4067
LSD [2]	—	0.013	0.054	—

[a-d] Means within a column without a common superscript differ (P ≤.05).
[1] Treatments:
1=Basal
2=Basal + PFA
3=Basal+PFA matrix, no PFA
4=Basal+PFA & PFA matrix
5=Basal+enzymes, enzyme matrices, no PFA
6=Basal+enzymes, enzyme matrics, PFA
7=Basal+enzymes, enzyme matrices, PFA   matrix, no PFA
8= Basal+enzymes, enzyme matrices, PFA matrix, and PFA

[2] Fisher's LSD value

Table 3. Cecectomized Rooster TME$_n$[1] and TAAD[2] Values *(Study 2)*

Treatment[3]	TME$_n$±SD	AATD±SD	TTD±SD	GATD±SD	PTD±SD	ALTD±SD	CYTD±SD	VATD±SD	METD±SD	ILTD±SD	LETD±SD	LYTD±SD
1	3128.05±105.86	88.15±4.26	83.19±4.05	92.29±3.50	88.72±4.05	84.29±6.09	76.20±15.09	87.36±6.03	92.45±5.40	88.05±5.73	90.58±4.10	90.62±3.70
2	3223.05±183.16	89.09±7.89	85.73±14.32	92.25±6.53	90.54±9.68	85.26±10.83	82.85±19.82	89.33±9.89	93.46±5.98	89.68±8.88	92.06±7.16	90.97±7.04
3	3022.96±265.38	87.93±5.65	85.86±7.83	92.43±3.55	90.77±4.55	84.68±5.99	81.13±15.53	89.72±5.84	94.99±3.13	89.36±5.53	91.80±4.51	90.70±4.15
4	3119.01±204.31	88.83±4.62	85.93±6.04	92.25±4.33	90.04±4.49	86.91±4.43	82.75±11.73	89.14±5.75	94.80±3.78	89.84±4.84	91.38±4.28	90.47±3.66
5	3101.79±218.84	86.58±6.38	83.76±9.43	90.78±5.23	86.69±8.31	80.24±10.31	78.25±18.36	85.53±8.40	92.93±4.29	86.62±7.35	89.55±6.07	88.12±6.53
6	3171.62±339.81	89.15±2.84	86.79±3.76	92.53±1.55	89.14±2.38	83.98±3.91	76.59±9.45	88.61±2.84	94.22±2.15	89.53±2.80	91.88±1.99	87.99±6.01
7	3179.82±267.87	88.74±5.86	82.45±7.71	92.40±5.77	88.13±8.17	86.35±9.02	74.80±21.39	87.76±8.44	95.15±3.40	89.54±6.54	91.20±5.76	88.95±814
8	2954.66±213.35	83.63±4.38	79.05±7.09	87.89±3.88	85.51±5.00	78.15±6.63	65.76±21.61	81.61±5.72	90.41±3.72	83.19±5.03	86.81±3.76	85.53±7.87
ANOVA P - Value	0.3598	0 .4812	0. 6063	0.4457	0.6822	0.2781	0.5691	0.3140	0.2981	0.3374	0.4333	0.6540
TRT 3 v. 4	0.4113	0.7403	0.9870	0.9358	0.8174	0.5557	0.8512	0.8670	0.9264	0.8739	0.8636	0.9420
TRT 7 v. 8	0.0575	0.0645	0.4060	0.05102	0.4082	0.0340	0.2968	0.0798	0.0230	0.0400	0.0792	0.2745

[1] True Metabolizable Energy corrected for Nitrogen
[2] AATD=Aspartic acid true digestibility; TTD=Threonine true digestibility; GATD=Glutamic acid true digestibility; PTD=Proline true digestibility; ALTD=Alanine true digestibility; CYTD= Cysteine true digestibility; VATD= Valine true digestibility; METD= Methionine true digestibility; ILTD= Isoleucine true digestibility; LETD= Leucine true digestibility; LYTD= Lysine true digestibility
[3] Treatments:

1=Basal
2=Basal + PFA
3=Basal+PFA matrix, no PFA
4=Basal+PFA & PFA matrix

5=Basal+enzymes, enzyme matrices, no PFA
6=Basal+enzymes, enzyme matrics, PFA
7=Basal+enzymes, enzyme matrices, PFA matrix, no PFA
8=Basal+enzymes, enzyme matrices, PFA matrix, and PFA

Appendix I.

Phosphorus Sparing Efficacy of diets containing Biostrong 510

Experimental Design
10 treatments replicated 9 times
Male broilers will be given a pretest from d1-d3
On d3 birds will be individually weighed and allocated to pens so that beginning pen weight will not vary
Experimental treatments will be fed on d3

Experimental Treatments
1. **Basal diet** @ 0.23% AP and 0.8%Ca[a]
2. **Basal diet** @ 0.28% AP and 0.8%Ca
3. **Basal diet** @ 0.33% AP and 0.8%Ca
4. **Basal diet** @ 0.38% AP and 0.8%Ca
5. **Basal diet** @ 0.23% AP and 0.8%Ca + phytase
6. **Basal diet** @ 0.23% AP and 0.8%Ca + Biostrong 510
7. **Basal diet** @ 0.23% AP and 0.8%Ca + phytase + Biostrong 510
8. **Basal diet** @ 0.28% AP and 0.8%Ca + phytase
9. **Basal diet** @ 0.28% AP and 0.8%Ca + Biostrong 510
10. **Basal diet** @ 0.28% AP and 0.8%Ca + phytase + Biostrong 510

[a]for all other nutrients formulation will meet Cobb Least Cost starter recommendations (no antibiotic).

Feed Manufacture
Diets will be fed in mash form.
Diets used for the standard curve will be created by manufacturing diet 1 and diet 2 as described above then blending diets to obtain diet 3 and 4. Diets 5, 6, 7, 8, 9, and 10 will come from adding product to the original diet 1 or diet 2 formulations. Phytase or Biostrong 510 will be added to a small 10 lb. allotment prior to being remixed with the total diet. Diet formulations will be similar to our past performance study.

Bird Models
Male Cobb 500 broiler chicks

Experimental Unit
One pen of 8, male Cobb 500 broilers

Variables Measured
- Starter diets will be manufactured and analyzed for the following prior to feeding
      a. Biostrong concentration
      b. Calcium
      c. Total Phosphorus

Experimentation
Broilers will be fed experimental diets from d3 to d21. Variables measured will include-
      a. Live weight gain
      b. Feed conversion ratio
      c. Tibia ash
      d. Available Phosphorus sparing effects can be calculated using assays a-c.
      e. Apparent metabolizable energy, digestible Ca, and digestible P will be estimated from total collection during d18-21.

Budget
Facility/Labor
      a. NA

Feed and Feed Manufacture
      a. Feed Manufacturing - $1,000
      b. Starter Feed – 250 lbs./trt. minimum of 2,500 lbs of feed @ $500/ton – $625

Chick cost
      a. 750 chicks @ $1.30 each - $975
      b. Transportation to and from commercial hatchery $300

Laboratory Analysis
      Prior to beginning the study
      a. Total Phosphorus – 10 feed samples @ 17/sample - $170.00
      b. Total Calcium – 10 feed samples @ 17/sample - $170.00
      c. Shipping to Delacon - ?

      AME, Digestible Ca and P
      a. 100 gross energy samples (feed and excreta) @ 36/sample – $3,600
      b. 90 calcium samples (excreta) @ 17/sample - $1,530
      c. 90 phosphorus samples (excreta) @ 17/sample - $1,530

Shipping/Transportation
      a. shipping of samples - $300.00

Total - $10,200.00 (plus shipping to Delacon for Biostrong analysis)

Total without the AME, Digestible Ca and P work = $3,440 (plus shipping to Delacon for Biostrong analysis)

Appendix II. Matrix Value Justification of a Commercially Available Phytase

Feed Manufacture:

Twelve dietary treatments, formulated to meet JBS United recommendations, were manufactured

at West Virginia University's pilot feed mill. Starter feed was formulated to meet Cobb Vantress

Inc. least cost starter recommendations and fed for a pre-test period of seven days. The basal

diet (Table 1), which totaled 348.98 lbs., was batched and divided into 12 aliquots with diet 1

totaling 26.43 lb., diet 2 totaling 27.53 lb., and diets 3 through 9 totaling 28.4 lb each (Table 2).

Diets 1, 2, and 3 were formulated as the available phosphorus standard curve diets and contained

.10% aP, .175% aP, and .25% aP, respectively. Potassium phosphate (KH_2PO_4) was

incorporated into diets 2 and 3 at .33% and .66%. Experimental phytase and corn starch were

added prior to individual mixing in a Hobart style mixer for ten minutes.

Broilers:

A total of 200 male, Cobb 500 broilers were purchased from a commercial hatchery at hatch.

One hundred ninety-two of these broilers were selected based on weight to create uniformity in

initial pen weight. Weighing occurred on day eight and groups of selected birds were randomly

allotted to 1 of 48 raised wire cages located in one, environmentally controlled room. Blocks of

treatments were comprised of 12 adjacent cages housing 4 birds per cage. There were four

blocks or replicates. Feed and water were provided ad libitum and birds received 24 hours of

light per day. During the 1-7d pre test period, birds were fed a nutritionally adequate starter diet

that met Cobb Vantress Inc. least cost starter recommendations. On d 8 each of the experimental diets were randomly assigned to cages within each block.

On d 21, birds were killed via cervical dislocation and final pen weight was obtained. The performance variables measured were beginning pen weight, ending bird weight, live weight gain, feed intake, feed conversion ratio, and mortality (Table 3). Left tibias were excised from each bird, dried, defatted, and ashed at 600^0 C for 16 hours to determine percentage ash (Table 4).

Statistical Analysis:

Analyses were completed using GLM ANOVA procedure from the SAS Institute and when significant differences were detected, treatment means were separated and compared using Fisher's LSD. Regression analysis was performed using standard curve treatments in order to obtain formulas to calculate phosphorus sparing.

Table 1. Basal Diet Formulation

Basal diet:

Ingredient	Working %	g/batch	lbs
Cornstarch	to 100		
Corn-finely ground	51.400	82240.00	181.15
SBM	39.690	63504.00	139.88
Soybean oil	5.000	8000.00	17.62
DL-Methionine	0.200	320.00	0.70
Limestone, ground	1.670	2672.00	5.89
Salt	0.400	640.00	1.41
Vitamin/Trace-mineral mix	0.625	1000.00	2.20
Bacitracin (BMD 60)	0.038	60.80	0.13
	99.023	158436.80	348.98

Table 2. Diet Formulations

Treatment	Treatment	Basal Amount (g)	KH$_2$PO$_4$ (g)	Corn Starch as Filler (g)	Treatment Total (g)
1	P-deficient corn-SBM basal diet (0.10% aP)	11,882.760	N/A	117.240	12,000
2	As 1 + 0.075% P (KH2PO4)	12,377.875	41.3	80.825	12,500
3	As 1 + 0.150% P (KH2PO4)	12,872.990	85.903	41.107	13,000

Treatment	Treatment	Phytase Inclusion Rate(g)	Basal Diet (g)	Corn Starch (g)
4	LF#1@250 U/kg	.418	12,872.990	126.592
5	LF#2@250 U/kg	.450	12,872.990	126.56
6	LF#3@250 U/kg	.432	12,872.990	126.578
7	LF#4@250 U/kg	.392	12,872.990	126.618
8	LF#4@500 U/kg	.785	12,872.990	126.225
9	11805@250 U/kg	.288	12,872.990	126.722
10	11803@250 U/kg	.291	12,872.990	126.719
11	Standard@250 U/kg	1.429	12,872.990	125.581
12	PF OL 23692@ U/kg	.746	12,872.990	126.264

Table 3. Broiler Performance

Treatment	Beginning Pen weight (kg)	Ending Bird Weight (kg)	Feed Intake (kg)	Live Weight Gain (kg)	Feed Conversion Ratio (kg/kg)	Mortality (%)	Tibia Ash (%)	Mg ash/chick
1	0.535	0.443^e	1.912^e	$.275^f$	1.78^f	18.75^b	20.20^g	308^h
2	0.536	0.603^c	2.815^b	$.469^{bc}$	1.50^{abcd}	0	26.99^b	549^{bc}
3	0.532	0.676^a	3.171^a	$.543^a$	1.46^a	0	32.39^a	764^a
4	0.533	0.513^{de}	2.390^{cd}	$.379^{de}$	1.58^{bcd}	0	21.82^{fg}	409^{fg}
5	0.538	0.510^{de}	2.379^{cd}	$.367^{de}$	1.62^e	0	21.65^{fg}	391^{fg}
6	0.522	0.601^c	2.588^c	$.460^{bc}$	1.57^{bcde}	6.25	24.36^{de}	443^{efg}
7	0.533	0.470^e	2.295^d	$.336^{ef}$	1.72^f	0	21.40^{fg}	381^{gh}
8	0.544	0.548^{cd}	2.565^c	$.412^{cd}$	1.56^{bcde}	0	24.67^{de}	459^{def}
9	0.533	0.552^{cd}	2.517^c	$.408^{cde}$	1.60^{de}	6.25	23.06^{ef}	446^{efg}
10	0.535	0.651^a	2.933^b	$.517^{ab}$	1.55^{abcde}	0	24.78^{cde}	531^{cd}
11	0.538	0.635^a	2.962^{ab}	$.500^{ab}$	1.48^{ab}	0	26.72^{bc}	621^b
12	0.525	0.619^{abc}	2.908^b	$.487^{ab}$	1.49^{abc}	0	25.17^{bcd}	505^{cde}
ANOVA P value	0.116	<0.0001	<0.0001	<0.0001	<0.0001	0.0038	<0.0001	<0.0001
LSD[1]		0.0729	0.2117	0.0727	0.0942		2.0228	72.758

[a-f] Means within a column without a common superscript differ (P≤.05)

1 Fishers Least Significant Difference

36

Table 4. Calculated Phytase Sparing Effects

Ending Bird Weight (kg)	TRT	Mean	Calculated aP	Sparing Effect
Slope= 1.55481	4	0.51256	0.135656447	0.035656447
Intercept= .30164	5	0.50122	0.128362951	0.028362951
	6	0.6012	0.192666628	0.092666628
	7	0.46497	0.105048205	0.005048205
	8	0.54771	0.158263711	0.058263711
	9	0.55187	0.160939279	0.060939279
	10	0.65091	0.22463838	0.12463838
	11	0.63446	0.214058309	0.114058309
	12	0.61859	0.203851274	0.103851274
Live Weight Gain (kg)	TRT	Mean	Calculated aP	Sparing Effect
Slope= 1.78497	4	0.37923	0.147178944	0.047178944
Intercept= .11652	5	0.36673	0.140176025	0.040176025
	6	0.45993	0.192389788	0.092389788
	7	0.33615	0.123044085	0.023044085
	8	0.41175	0.165397738	0.065397738
	9	0.40761	0.163078371	0.063078371
	10	0.51726	0.224507975	0.124507975
	11	0.50006	0.214871959	0.114871959
	12	0.48725	0.207695367	0.107695367
Feed Conversion	TRT	Mean	Calculated aP	Sparing Effect
Slope= 1.96006	4	1.57691	0.177743861	0.077743861
Intercept= -2.15563	5	1.6232	0.156269861	0.056269861
	6	1.56934	0.181255596	0.081255596
	7	1.72217	0.110357529	0.010357529
	8	1.55969	0.185732245	0.085732245
	9	1.59543	0.169152406	0.069152406
	10	1.54916	0.190617128	0.090617128
	11	1.48201	0.221768114	0.121768114
	12	1.49301	0.216665198	0.116665198

Tibia Ash	TRT	Mean	Calculated aP	Sparing Effect
Slope= 81.25	4	21.82	0.117058462	0.017058462
Intercept= 12.309	5	21.65	0.114966154	0.014966154
	6	24.36	0.14832	0.04832
	7	21.4	0.111889231	0.011889231
	8	24.67	0.152135385	0.052135385
	9	23.06	0.13232	0.03232
	10	24.78	0.153489231	0.053489231
	11	26.72	0.177366154	0.077366154
	12	25.17	0.158289231	0.058289231
Mg ash/chick	TRT	Mean	Calculated aP	Sparing Effect
Slope= 3037.5	4	409	0.131802469	0.031802469
Intercept= 8.65	5	391	0.125876543	0.025876543
	6	443	0.142995885	0.042995885
	7	381	0.122584362	0.022584362
	8	459	0.148263374	0.048263374
	9	446	0.143983539	0.043983539
	10	531	0.171967078	0.071967078
	11	621	0.201596708	0.101596708
	12	505	0.163407407	0.063407407

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Education:

West Virginia University, Morgantown WV   Aug. 2010
Masters of Science Animal Nutrition
GPA: 3.5/4.0

West Virginia University, Morgantown WV   May 2008
Bachelor of Agricultural Sciences
Major: Animal and Nutritional Sciences
Minor: Horticultural Studies
Major GPA: 4.0/4.0
Cumulative GPA: 3.6/4.0

Experience:

West Virginia University, Morgantown WV   August 2008-Present
Graduate Research Assistant
- Feed production, WVU Feed Mill
- Feed Formulation
- Proximate Analysis
- Tibia Ash
- Assisting in Multiple Research Studies
- Teaching Aid for Poultry Science Labs
- Teaching Assistant for Collegiate Poultry Judging

USDA-ARS, Beaver WV   May 2006-August 2008
Biological Sciences Aide
- Basic Animal Husbandry
- Fecal Egg Counts
- Packed Cell Volume Counts
- Crude Fiber Analysis of Forage Grasses
- Vaccinations, De-worming and Blood Draws

- Basic Fertilizer Application Calculations

Dairy Queen, Fairlea WV   June 2002-Decemeber 2006
Shift Manager
- Daily Engagement with Customers and Public
- Managed Multiple Employees
- Performed Inventory Tasks
- Responsible for Multiple Financial Tasks

Coursework: I have completed the following animal science related courses: Introduction to Animal Science, Companion Animal Science, Introduction to Animal Nutrition, as well as Ruminant Nutrition, Non-ruminant Nutrition, Poultry Production, Animal Physiology, Animal Parasitology, Nutritional Biochemistry, Calving Management, and Collegiate Poultry Judging.

Projects: **USDA-ARS Projects:**
- "Plant Materials for Control of Gastrointestinal Parasites in Small Ruminants"
- "Meat Goat Finishing Systems for Appalachian Small Farms"
- "Medicinal Botanical Production and Processing"
- "Native Plant Resources for Small Ruminant Production in Appalachia".

Graduate Projects:
- West Virginia University Poultry Research Presentation-West Virginia State Fair

- "Justifying phytogenic feed additive matrix values in conjunction with exogenous feed enzymes".

- "Modeling the effects of high pellet quality on broiler growth and carcass quality".
- "Increasing mixer-added fat improves exogenous enzyme efficacy and broiler performance".
- "Examining the relationship between pellet quality, broiler performance and bird sex".
- "Thermostability and efficacy of exogenous feed enzymes".

Skills: Computer: Microsoft Office, Brill Feed Formulation Program, Taurus Beef Cattle Ration Formulation and Evaluation Software, R Statistical Software, Alphanumeric Entry of 65 wpm.
Personal: Certified in CPR and First Aid, Certification in Avian Influenza Task Force Training, Multiple presentations at National meetings, Feed

conversion ratios and other calculations, Driven, Hard Working, and Cooperative

Achievements: Promise Scholar
Magna Cum Laude graduate.

Activities: Poultry Science Journal Club
Gamma Sigma Delta
Collegiate Poultry Judging Coach
Research assistant to VA Poultry Grower's Co-op
Poultry Science Association