Setting and Meeting Standards for the Replacement of Pronutrient Antibiotics in Poultry.

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Introduction

Notwithstanding vigorous and, at times, intense on-going debate on its merits and justification, the replacement of veterinary prescription-free pronutrient antibiotics used in poultry production gathers momentum, albeit at different rates regionally around the world.

To serve as background to this communication, the text of a recent review of antibiotic replacement is attached as an Appendix (Rosen, 2003a). Following a brief summary thereof, the results of two recent studies are presented on (i) current problems and thinking in the field of antibiotic alternatives and (ii) the development and application of comprehensive multi-factorial models to quantify productivity losses and to assess the efficacy of antibiotic substitutes. Two main questions are addressed.

What are current views on the status, methodology, problems and future needs in the setting and meeting of antimicrobial replacement standards, as seen internationally by suppliers, users, consultants, educators/communicators and academics?

What contribution can comprehensive empirical nutrition response models from multi-factorial regression analysis of all available data make, now and in the future, in setting and meeting standards for penalty-free antibiotic replacement by alternative additives and management systems?

Current Perspectives

In the first place there are many different categories and literally hundreds of products available world-wide on offer for use alone or in admixture and/or with changed feed formulation and managemental adjustments. Thousands of publications are relevant for appraisal and the literature is expanding rapidly.

Terminology currently used in this field leaves much to be desired. Some descriptors are vague and inaccurate in scientific use and untransparent to consumers. The oft-criticised regulatory authorities have, at least, been wise, for example, in electing for 'micro-organism' or 'direct-fed microbial' and for 'oligosaccharide' rather than 'probiotic' and 'prebiotic'. Feed additives can be precisely nominated and transparent as duplex descriptors, specifying nature and function, e.g., pronutrient bacitracin, prophylactic narasin, therapeutic penicillin and pro-environmental phytase (Rosen, 2003b). All four have nutritive effects. The term 'non-nutritive' for additives should be discarded. It is incorrect because pronutrients can mitigate limiting nutrient insufficiencies, with or without supplemental addition of essential nutrients *per se*.

The effects of admixture, sometimes misrepresented as synergistic, can be better specified as sub-additive, additive, synergistic, ineffective or antagonistic, when, for example, two plus three provides four, five, six, two or three and one unit(s) of response respectively and responses to antimicrobials and alternatives need routinely to be specified in absolute units in place of or together with percentages in scientific and commercial documentation.

Many excellent reviews focus mainly on gut and other health aspects, modes of action, intermediary metabolism and floral/host interactions (Apajalahti, 2003; Bedford & Fothergill, 2002; Ferket & Gernat, 2002; Huyghebaert, 2003; Klein-Hessling, 2001). These facilitate the introduction and development of new products, but metabolic indicators and modes, as against amounts, of action, in isolation, do not quantify nutritional responses for practical application. The use of *in vitro* or *in vivo* mechanistic indicators in diet formulation persists more as an art than a science. Laboratory data and metabolic indicators urgently need significant *in vivo* corelationships for deployment in praxis.

The world literature contains an abundance of controlled test reports on a host of feed additive antimicrobials and alternatives offered to improve poultry production efficiency. Appendix Tables 3, 4 and 5 contain a preliminary comparison of exogenous enzymes and antibiotics. The setting of replacement standards can be greatly facilitated by the use of comprehensive empirical nutritional models for the diverse replacement conditions in mind, but meeting such standards is handicapped at present by the lack of analogous models for alternatives, save perhaps for enzymes, such as non-starch polysaccharidases plus or minus phytases.

Reviews to date suggest that exogenous enzymes are the most promising replacements for antimicrobials in poultry, with lesser indications for microbials and oligosaccharides. As yet it is difficult in poultry to rate acids and botanicals in the pecking order of alternative categories on offer. The substantial coefficients of variation seen in nutritional responses evident in medium-sized and larger data collections illustrate the potentials hazards in relying on a handful of five or less controlled test results.

As guidelines for future research and development, the views of diverse interested parties on the evaluation of antibiotic substitutes have recently been studied.

Opinion Survey

A pilot survey was devised to assess opinion on the current status and future needs in substitution of in-feed antimicrobial additives. Fifty individuals were asked seven questions (Table 1). There were five categories of 10 interviewees comprising (i) substitute suppliers, (ii) users, (iii) consultants, (iv) educators/communicators and (v) academic researchers. The questions were intended to range broadly over efficacy measurement and comparisons, current problems, key determinant factors, test replication, present candidate relativities and posology. The answers revealed a wide variety of 74 topics, many interconnected, listed in Table 2.

Question 1. Nineteen of the 40 respondents, excluding educators/communicators, strongly preferred to conduct their own tests for decisive action. Fourteen of the 50 required better information on poultry health status and/or specification of known disease or environmental challenge, whilst 12 regretted the lack of presentation of all available relevant data. In the total of 118 comments there were no striking differences between the five interviewee groups. Single references were made to effects of gut pH, histological effects, immune status, the need to include anticoccidials, dietary grain types and qualities and to being 'limited to progress by trial and error'.

Question 2. More than half of the respondents (64%) made no distinction between comparisons of substitutes and substitutes versus antibiotics. More than ten divergent criteria for response measurement had no consensus in specific zoological or economical end points. As to methodology, the most prevalent concern was absence of negative controls, followed closely by difficulties in translating results from research conditions into practice. There was accord, nonetheless, in the need for more detailed guidance on effects on carcass and produce quality, dose-response relationships, alternatives modes of application and summary comparisons of antibiotic and substitute efficacities. Otherwise differences in criteria and methodology between suppliers, users, consultants, communicators and academics were few.

Question 3. The 50 participants nominated a total of 92 specified problem areas, including variation in response (13), uncontrolled tests (8), sub-standard test designs (8), no mode of action support (5), no feed composition (4), high own-test costs (4) and invalid field test data (4).

Question 4. This elicited 101 items in which other factors influencing choice of substitute, not previously mentioned, were price and economics (10), supplier reputation (10), regulatory authority status (6), ill-supported product claims (5), other users' opinions (5), retailer and food consumer views (4) and active ingredient stability in store and in feed (3).

Question 5. Regarding the minimum number of tests required for their substitution decision, five interviewees 'had no idea' and three deemed this question unanswerable. The 42 numerical responses varied considerably, ranging from 2-50, with a mean of seven (c.v. 120%). At the lower end (two), tests had to be 'own design'. The higher values (50, 33) were from experienced researchers in the evaluation of pronutrient feed additives. Three tests satisfied 11 respondents, 11 opted for five and 7 needed 10. Average test numbers required ascended through suppliers (4), users (5), consultants (7), communicators (10) and academics (10). The numbers chosen by suppliers, users and communicators varied least (cv 40-48%), compared with consultants (133%) and academics (161%).

Question 6. One interviewee was unable to offer efficacy ratings for the nominated six categories of antibiotic substitute. Only one respondent rated poultry and pigs separately. The overall panel appraisal of efficacy in descending order was enzymes, acids, microbials, botanicals, oligosaccharides and others. The proportions for ratings in positions 1-6 respectively were 44%, 32%, 28%, 32%, 32% and 68%. Of the five interviewee groups, four of them placed enzymes as best, three placed acids second, two placed microbials third, two placed botanicals fourth, three placed oligosaccharides fifth and all placed others sixth. In the others category, 32/50 had no specific type in mind (seven suppliers, seven users, six consultants, six communicators and six academics), whilst six specified nutrients, four chemicals (copper sulphate, zinc oxide, arsenicals and chelates), three antibodies and one each antioxidant, bacteriophage, betaglucan, betaine, peptide and polysaccharide.

Question 7. Discussion of posology elicited 66 observations from consultants (17), suppliers (15), users (12), communicators (12) and academics (10). These comprised 24 utilising only own tests in assessing zoologic dose-responses, economic dose-responses and economic optima. Of the 18 basing decisions on suppliers recommendation, three insisted on dose-response data and three sought a x0.5/x1/x2 demonstration, related to any single recommended value. Other individual viewpoints required FDA approval, challenge specification, minimum dosage of 10^7 c.f.u./g feed, and three just proffered 'guesstimation', 'don't know' and 'not my job'.

The wide scope encountered indicates a need for a second phase to help pinpoint and quantify and also to prioritise future research and development programmes.

Nutritional Models

The elaboration and application of comprehensive multi-factorial empirical models, based on the results of all available pronutrient response data, containing genetic, environmental, managemental, dietary, temporal and posological variables, with interactions, is crucial in harnessing feed additives to improve productivity. Table 3 contains a selection of relevant independent variables of proven importance in modelling to date, centred mainly on antimicrobials, enzymes and microbials. Most of these variables have been significant to greater and lesser extent. Models containing up to 20 or more significant variables can account for up to 70% of variations of nutritional responses in time and place (Rosen, 2003c). The system is limited only by researchers' failure to measure and report fundamental key variables, such as temperature, altitude, lighting pattern, etc.

Hitherto, one has presumed arbitrarily that a minimum of about 50 controlled test results is required to take first account of major influential independent variables. In order to investigate more precisely how many tests might be required for the elaboration of useful models, an exploratory test has been made, based on pronutrient antibiotics. The latter resource contains 1,709 feed intake, live weight gain and feed conversion responses with 708 mortality, including bacitracin zinc (640), bacitracin methylene disalicylate (194), chlortetracycline (299), oxytetracycline (247) and virginiamycin (329). Random selection provided 20 sub-sets of 86/85. Known major contributors to variation were tested, viz, levels of control performance, FDIC, LWGC, FCRC, MORTC, duration of test, DUR, year of test, EXDAT, logarithmic dose, log(PPM+1), use of anticoccidial, COC, and presence of diagnosed or endemic disease, VET.

Table 4 contains 'Bromycin' feed intake, live weight gain, feed conversion ratio and mortality effect models for the full data set, with 4, 6, 5 and 4 significant independent variables respectively. Randomly-selected sub-sets, each containing 85 or 86 tests, provided 20 analogous sets of models, the main characteristics of which are summarised in Table 5.

Feed intake effect. The parent model has significant variables in control performance, duration, year of test and anticoccidial. Only 12 of the sub-sets yield models. These contain only 18 significant terms (38%) in toto out of a possible 48. There are four FDIC, two DUR, three EXDAT, one log(PPM+1), five COC and three VET. For FDIeff, samples of 85 tests fail to furnish a model akin to the parent (n = 1,709), though, interestingly, one includes a significant log term, not found in the parent model.

Live weight gain effect. For LWGeff, all 20 sub-sets afford models with 1-4 significant independent variables. The significant variables, 37/120 (31%), contain two LWGC, four DUR, two EXDAT, six log(PPM+1), five COC and 18 VET. The suggestion therein is that VET alone might emerge in smaller data sets than 85. The range in partial regression coefficients for VET is +60 to +141g, having a mean of +92g (c.v. 24%), compared with the parent +76g. Two models approach the parent model, each revealing four of its six variables. But only two in 20 possibles reveals the limitations of n = 85.

Feed conversion ratio effect. The FCReff sub-sets also have a full complement of 20 models with 1-5 significant terms, in only one of which all five are represented, as in the parent model. R^2 and SD of the fragment models range from .068 to .653, with standard deviations from .0686 to .126. Total significant term appearances are 54/120 (45%), including FCRC 18, DUR 15, VET 10, log(PPM+1) six, EXDAT four and COC one. There are large differences in partial regression coefficients, ranging FCRC from -.053 to -.252, DUR from .0019 to .0056 and VET from -.088 to -.308. As with LWGeff, n = 86 is manifestly restrictive.

Mortality effect. The numbers of cases for the mortality sub-set models number 29 to 42 (mean 35). All 20 models contain significant terms, with R^2, .215-.912, and SD, 1.74-4.83. Total significant terms are 30/120 (25%), comprising MORTC 18, VET seven, COC two, log(PPM+1) two and DUR one. The partial regression coefficients of the dominant MORTC term range from -.188 to -.959 with an average of -.596 (c.v. 38%), cf. -.647 in the parent model. Three models contain three significant terms, each having MORTC and VET with COC 2/3 and log(PPM+1) 1/3.

The observed inferior sub-set models for FDIeff can be anticipated in the light of its high coefficient of variation (768%) in 1,709 tests, as compared with MORTeff (404%), FCReff (162%) and LWGeff (125%). Further studies are in progress (i) to elucidate the potential values of a broader range of sub-sets, i.e. 43, 85, 171, 244, 342 and 427 for Bromycin and (ii) to compare the outcome for the largest single resource geared solely to enteric activity, bacitracin, (n = 840), for a sub-set range of 35, 70, 105, 140 and 210 tests.

Synopsis and Prognosis

Setting standards for the substitution of individual antibiotics is already endowed with working nutritional models based on decades of research. Meeting such standards is easier for those exogenous enzymes already modelled, when used singly, with a need next to model admixtures. Other substitute candidates lag behind, but literature to date appears to offer potential for the elaboration of multi-factorial models for acids, microbials and maybe oligosaccharides.

Efficient substitution of antibiotics at present is complicated by the large number of products on offer, dissimilarities in candidate categories, superficial test programmes in bridging the gap between research conditions and praxis and by a broad, vague spectrum of user targets.

Pro tempore, then, enzymes have a lead in the shorter term. For the rest, we seem, perforce, to progress, in the words of one researcher interviewed, mainly 'by guesstimation, trial and error'. Elaboration of working models for substitutes is, as yet, embryonic, but it proffers a broad and challenging field for research in the years ahead towards an ultimate goal of antibiotic replacement penalty-free.

References

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Table 1. Questionnaire on the current status and future needs of antibiotic feed additive substitutes

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1. By what method(s) do you compare the efficacy of a substitute with that of an antibiotic feed additive?

2. By what other method(s), if any, do you compare the efficacies of two or more substitute candidates?

3. What problems do you meet in 1 and 2?

4. What other factors, if any, determine your choice of an antibiotic substitute?

5. What minimum number of valid tests do you need to measure the substitute's zoologic and economic values?

6. In what descending order of efficacy do you rate as antibiotic substitutes: (a) acids; (b) botanicals (plants or derivatives); (c) enzymes; (d) microbials; (e) oligosaccharides; and (f) others?

7. Having made a choice, how do you decide what dosage to use?

Table 2: List of topics raised and discussed relating to the seven Table 1 questions on selection, dosage and efficacy of pronutrient antibiotic substitutes

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1. Disparate product types
2. Large numbers of products
3. Product qualities
4. Activity definition
5. Ill-defined admixtures
6. Product uniformity/quality control
7. In-feed analysis
8. Botanicals; source and process
9. Storage stability
10. Processing thermostability
11. Application technology
12. Supplier calibre
13. Promotional bias/selected data
14. Uniquity
15. Diverse response criteria
16. Feed consumption
17. Live weight gain
18. Feed conversion efficiency
19. Mortality
20. Carcass qualities
21. Environment improvements
22. Palatability
23. Water intake
24. Litter/bedding quality
25. Response frequencies
26. Nutrient equivalence(s)
27. Selected test data
28. Genotype influence
29. Lighting pattern
30. Key independent variables
31. Dose/response zoologic
32. Dose-response economic
33. Recommended single dose x0.5/x1/x2
34. Variations in responses
35. All-inclusive bibliographies
36. Insufficient test results
37. Efficacy test designs
38. Own tests
39. High test costs
40. Small animal test numbers
41. Inadequate replication
42. Statistical methods
43. Inappropriate test conditions
44. No negative controls
45. Too small-scale tests
46. No feed/nutrient composition
47. Within-test comparison
48. Few independent assessments
49. Nutritional response models
50. Annual economics
51. Unaccounted variability
52. Research versus praxis
53. Extreme deficiency conditions
54. Invalid field test designs
55. Product admixture interactions
56. User opinions
57. Retailer/consumer influences
58. Regulatory status
59. Tissue residues
60. Safety data
61. Nutrition/environment interaction
62. Nutrition and/or prophylaxis
63. Challenge definition
64. Disease status
65. Clostridia
66. Necrotic enteritis/coccidiosis

67 Diarrhoea

68. Heat stress
69. Mode of action/response relations
70. Enteric ± systemic activities
71. Gut floral activities
72. In vitro support data

73 International differences
74 Regional adjustments

Table 3. Independent genetic, environmental, managemental, dietary and nutrient variables used in the elaboration of nutritional models

control performance
duration
year of test
dose
initial age
not day-old
sex
phased dose
factor 2 dose*
selected birds
housing
stocking density

feed process
antibiotic
anticoccidial
antihistomonial
metabolic test
diet marker
part-purified diet
disease challenge
favson
institute test
country
brand

maize**
sorghum
wheat
barley
oats
rye
animal fat
vegetable oil
fish meal
meat/bone meal
wheat offal
rice bran

gross energy**
net energy
crude protein
crude fat
crude fibre
calcium
phosphorus
lysine
methionine
meth.+cyst.
threonine
tryptophan

* second antibiotic/enzyme/acid/microbial/other pronutrients
** as dietary concentration (columns 3 and 4)

Table 4. 'Bromycin' (antibiotic) broiler models

FDIeff	= - 79.5	- .0380FDIC	+ 2.98DUR	+ 1.02EXDAT*		- 24.3COC	
R^2 .052	se 38.7	.006	.507	.465		7.07	
SD 126	p .029	.000	.000	.028		.001	
n 1709							
LGWeff	= - 72.4	- .0114LWGC	+ 1.02DUR	+ .871EXDAT	+ 19.5log(PPM+1)	- 16.8COC	+ 76.1VET
R^2 .171	se 15.0	.004	.153	.183	3.22	2.70	5.56
SD 47.4	p .000	.009	.000	.000	.000	.000	.000
n 1709							
FCReff	= .301	- .161FCRC	+ .00306DUR	- .00159EXDAT	- .0286log(PPM+1)		- .116VET
R^2 .287	se .029	.007	.000	.000	.007		.012
SD .100	p .000	.000	.000	.000	.000		.000
n 1709							
MORTeff	= - 1.13	- .647MORTC	+ .0363DUR		+ 1.08log(PPM+1)		- 2.12VET
R^2 .685	se .805	.021	.012		.336		.475
SD 3.12	p .000	.000	.003		.000		.000
n 708							

* year - 1900

Table 5. Summary characteristics of parent and random sub-set nutritional models

Variable	n	Control	Effect	DUR (days)	EXDAT	log(PPM +1)	COC	VET	No. of variables	R^2	SD
PARENT											
Feed intake (g)	1709	2832	16.9 (0.6)*	48.0	69.3	1.26	.409	.046	4	.052	127
Live weight gain (g)	1709	1240	41.4 (3.4)	48.0	69.3	1.26	.409	.046	5	.171	47.7
Feed/gain	1709	2.286	-.073 (3.2)	48.0	69.3	1.26	.409	.046	6	.287	.101
Mortality (%)	708	5.46	-1.41 (26)	52.5	71.1	1.26	.523	.089	4	.679	3.15
SUB-SET MEANS											
Feed intake (g)	85.5	2832	16.9 (0.6)	48.0	69.3	1.26	.429	.046	1	.105***	124***
CV** (%)	0.6	4.6	61	2.6	0.7	2.2	10	36	105	61	18
Live weight gain (g)	85.5	1240	41.4 (3.4)	48.0	69.3	1.26	.409	.046	1.9	.175	47.5
CV (%)	0.6	4.6	11	2.6	0.7	2.2	10	36	57	51	11
Feed/gain	85.5	2.286	-.073 (3.2)	48.0	69.3	1.26	.409	.046	2.7	.286	.099
CV (%)	0.6	1.6	14	2.6	0.7	2.2	10	36	34	47	16
Mortality (%)	35.4	5.46	-1.41 (26)	52.5	71.1	1.26	.523	.089	1.5	.618	2.82
CV (%)	11	22	78	1.5	1.7	22	14	40	51	40	30

* as % of control ** CV coefficient of variation *** n = 12