Combination of Therapeutic Agents

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The primary reason for combining therapeutic agents in feeds is to achieve broader control of conditions to which animals are exposed. The need for these combinations is evident in modern husbandry because of large animal units which require mass preventive medication. To clear combinations, data relating to efficacy, tissue residue levels and elimination rates, and agent stability in premixes and feeds must be shown for each agent and for each combination. FDA regulations do not permit the alteration of a combination of agents after approval has been granted. Thus the combinations that are cleared should contain the most efficacious agents available and have wide application. Data have been presented which showed that blood and tissue levels were the same when an agent was used alone as when combinations of agents were used, indicating that induced disease experiments could be eliminated without sacrificing knowledge of efficacy for an agent.

THE COMBINING of agents in feed formulations is not a new concept to the feed industry. The blending of proteins from different sources to obtain a more desirable amino acid balance is a well-established practice. Other feedstuffs are similarly combined in modern rations to give maximum production. It is not surprising, therefore, that as chemotherapeutic agents became available for use in feed they were combined to broaden the spectrum of coverage and increase animal performances. Available therapeutic agents show their effect by accelerating growth, increasing production, or controlling disease—e.g., coccidiosis, blackhead, parasites. Because of the need to obtain these effects for economical animal production, there is real reason for combining several therapeutic agents in a feed. However, when approval for combination use of agents is sought, the industry is confronted with several problems which include choosing the therapeutic agents that are to be used in a combination from the great number available; satisfying the data requirements, which have increased since the Food Additive Amendment became effective; and obtaining FDA approval for combinations so that the needs of the feed industry can be met. The number of possible combinations of therapeutic agents are many. At the present time, there are 53 agents that have been cleared individually for addition to feeds. These are listed by the number that may be used for each class of livestock: chickens, 42; turkeys, 38; swine, 27; sheep, 6; dairy, 6; beef, 8.

Some of the 53 agents may be used in all classes of animals, while others are restricted for a specific class and purpose (*2*). In some cases, the agent has been cleared for use by itself, and in other cases, in various combinations. A single agent may be used for more than one purpose.

There are large numbers of possible combinations. A hypothetical case will illustrate the complexity of the situation. If, for one animal, four agents are desired in a feed to control four conditions, it is necessary to study the effect of each agent singly and in all the possible combinations. Generally, there are four distinct compilations of data necessary to satisfy FDA needs: the tissue residues present and the rate of elimination of these residues; drug agent stability of the combined drugs in premixes and finished feeds; and maintenance of efficacy of each agent in the possible combinations. In addition, it is often necessary to obtain confirmatory efficacy data from an outside source. Thus, 16 different treatments must be tested in a minimum of two or three experiments with animals, and an inestimable number of assays must be conducted in the laboratory.

This hypothetical case is an illustration that complex combinations require considerable work and time for clearance. Furthermore, once the combination has been approved, FDA regulations dictate that it be used as an intact unit, at the specified levels. Alteration of levels, or deletion or addition of an agent will require complete repetition of all experimental work for clearance.

It would be simpler to take a negative stand and not attempt FDA clearance of combinations of agents because of the complex regulations. However, progress would not be made with this attitude, nor would our obligations to the animal industry be met. Experience has established the need for more than one agent in a feed to achieve the most economical production in livestock and poultry.

Choice of Agent and Responsibility for Clearance

Before work on clearance of a combination can begin, the choice of agents must be made and the responsibility for initiation of the project delineated between the different companies whose products are involved. The choice of agents to be cleared is a problem in itself. Random selection of agents is an unlikely approach because the chance of choosing a less effective agent is great. In some instances, certain agents will be cleared in combinations to meet the needs for an area problem. In others, the choice is made to meet a competitive situation or to have something "new." Many feed manufacturers routinely test agents alone and in combination and make a choice of agents based on performance. When a new product is introduced that covers a different condition or is superior to an old product, it receives consideration for clearance in combination with established products.

Whatever the reason for choosing agents to be cleared in combination, the combinations must have wide application to justify the expense. It would appear, too, that the less effective agents should be screened out and only the most effective agents, for each specific purpose, considered for combination use. Thus, it is evident that someone must make a choice, and it must be made judiciously. Under the economic stresses of animal production, it is essential that the best possible use be made of the available products so that their combined use may broaden or enhance, or more effectively cover the conditions to which animals are exposed.

Once the agents are chosen for combination use, it is necessary to determine who is responsible for experimental work. If one company supplied the best agents

Table I. Influence of Zoalene on Efficacy of Chlortetracycline (CTC) in Artificially-Infected Broilers with Infectious Synovitis[a]

Zoalene, %	Treatment CTC, Grams/Ton	Infected	No. Dead/ No. Infected	No. Lesions/ No. Survivors
0	0	No	0/30	0/30
0	0	Yes	9/30	3/21
0	200	Yes	0/28	0/28
0.0125	0	Yes	7/30	1/23
0.0125	200	Yes	0/30	1/30

[a] Three replicated groups of 10 straight-run White Rock broilers, 6 weeks of age. Medication started on day of inoculation and continued for 14 days.

Table II. Influence of Zoalene on Levels of Chlortetracycline (CTC) in Chicken Blood and Tissues[a]

Treatment			Tissues, μg./Gram				
			Muscle				
Zoalene, %	CTC, Grams/Ton	Blood, μg./Ml.	Dark	Light	Fat	Liver	Kidney
0.0125	200	0.16	0.05	0.09	0.02	0.15	1.00
		0.14	0.07	0.08	0.05	0.14	1.10
		0.13	0.05	0.07	0.04	0.10	0.65
		0.19	0.07	0.07	0.02	0.19	0.90
		0.20	0.07	0.10	0.05	0.17	1.00
	Av.	0.16	0.06	0.08	0.04	0.15	0.95
0	200	0.19	0.05	0.09	0.09	0.12	0.90
		0.15	0.09	0.09	0.05	0.19	0.90
		0.19	0.07	0.11	0.09	0.15	0.80
		0.24	0.08	0.15	0.10	0.22	1.23
		0.18	0.08	0.11	0.03	0.14	0.85
	Av.	0.19	0.08	0.11	0.07	0.16	0.94

[a] Samples taken while on medicated diets; tissues received from Dow Chemical Co.

Table III. Influence of Penicillin and Sulfamethazine (SM) on Levels of Chlortetracycline (CTC) in Swine Blood and Tissues[a]

Treatment, Grams/Ton			Blood,	Tissues, μg./Gram			
SM	Penicillin	CTC	μg./Ml.	Muscle	Fat	Liver	Kidney
0	0	100	0.16	0.23	0.04	0.60	1.20
			0.23	0.30	0.11	0.82	1.45
			0.21	0.34	0.04	1.40	0.85
		Av.	0.20	0.29	0.06	0.94	1.13
100	0	100	0.15	0.23	0.04	0.60	0.98
			0.13	0.14	0.02	0.61	0.76
			0.10	0.11	0.03	0.67	0.83
		Av.	0.13	0.16	0.03	0.63	0.86
100	50	100	0.14	0.17	0.07	0.80	0.94
			0.21	0.21	0.05	1.35	1.15
			0.16	0.28	0.06	0.80	0.94
		Av.	0.17	0.22	0.06	0.98	1.01

[a] Samples taken while on medicated diets after 28 days.

Table IV. Influence of Penicillin and Chlortetracycline (CTC) on Sulfamethazine (SM) Levels in Swine Blood and Tissues[a]

Treatment, Grams/Ton			Blood,	Tissues, μg./Gram			
SM	Penicillin	CTC	μg./Ml.	Muscle	Fat	Liver	Kidney
100	0	0	3.87	0.50	0.86	0.92	2.51
			3.87	1.07	0.62	1.07	3.94
			2.59	1.10	—	1.20	2.69
		Av.	3.44	0.89	0.74	1.06	3.05
100	0	100	3.39	0.59	0.62	1.75	3.19
			2.69	0.95	0.53	1.50	2.48
			6.11	1.01	0.60	2.13	2.34
		Av.	4.06	0.85	0.58	1.79	2.67
100	50	100	2.99	0.68	0.56	2.05	3.19
			4.79	1.51	0.79	0.85	4.08
			2.51	1.12	0.53	1.43	3.03
		Av.	3.43	1.10	0.63	1.44	3.43

[a] Samples taken while on medicated diets.

for all conditions, the responsibility for clearance would obviously be less of a problem. However, this is unlikely. When a new agent is ready for market, it appears that major responsibility rests with that supplier. Where established agents are involved, clearance often becomes a cooperative effort. In this case, as many as four companies, each furnishing data for their agent, may be cooperating.

Each company must determine whether its agents, singly or in the various combinations, will alter tissue residues and clearance rate; agent stability in premixes and finished feeds; and efficacy of each agent in the possible combinations. After these data have been accumulated, the initiator must submit a Food Additive Petition to the FDA, delaying production until approval has been obtained.

Compatibility of Agents

A third major point for consideration is compatibility of agents and a means for keeping data requirements for clearing combinations at a minimum, without sacrificing knowledge of efficacy.

As experience has been gained in testing combinations of therapeutic agents, there appears to be a clear-cut principle evolving that will reduce the work and time required for clearance. This principle is that efficacy and noninterference of agents in combinations are indicated when blood and tissue levels resulting from feeding the single agent are equal to those resulting from feeding the combined agents. While this observation was noted in work done with chlortetracycline, the same principle may apply to other therapeutic agents, especially those known not to affect the physiology of the animal.

Two examples are presented to illustrate the principle. The first example is from two experiments used to clear the combination of zoalene (3,5 dinitro-*o*-toluamide) and chlortetracycline. It has always been necessary to prove that efficacy of chlortetracycline is maintained by conducting an experiment to show that an artificially-induced disease is controlled by this antibiotic in the presence and absence of other agent(s) used in the combination. Data presented in Table I demonstrate that the efficacy of chlortetracycline was maintained when zoalene was present in the rations of broilers, and the birds were artificially infected with infectious synovitis (*3*). Obviously, zoalene was ineffective in reducing mortality from synovitis as its effect is not antibacterial. To show efficacy, an organism is chosen that is known to be sensitive to one agent of the combination, but not to the other(s).

The second experiment fulfills the requirement to demonstrate that blood

and tissue levels are not increased or clearance time delayed. In Table II, the data illustrate that the presence of zoalene did not alter the blood and tissue levels of chlortetracycline. Nor was there an effect on the comparable clearance time, although these data are not shown. As in the previous experiment, an organism, *Bacillus cereus* var. *mycoides*, known to be sensitive to chlortetracycline and not to zoalene, was used as the assay organism. Thus, the same conclusions may be drawn from the two experiments—namely, efficacy was maintained and compatibility or noninterference of agents was observed. Conversely, the presence of chlortetracycline and other commonly used antibiotics in the diets of chickens did not alter the blood and tissue levels of zoalene (*1*). Similar observations have been made with other agents, such as nystatin, N.F.-180, and hygromycin in broilers, and reserpine and nystatin in turkeys.

Another example of compatibility involves three antibacterial agents. The data in Table III illustrate the similarity of levels of chlortetracycline in blood and tissues when groups of pigs were fed rations containing chlortetracycline; chlortetracycline and sulfamethazine; or chlortetracycline, penicillin, and sulfamethazine. The data in Table IV similarly illustrate that sulfamethazine levels in swine blood and tissues were not affected by the feeding of other agents. The differences between the average values in the tables are within the expected range of biological variation. The data on the influence of the other two agents on penicillin blood and tissue levels are not shown because the levels were zero. Tests have also shown that hygromycin and nystatin do not affect the efficacy or blood and tissue levels of chlortetracycline in swine.

Thus, the author's experiences indicate that interference with the efficacy or blood levels of chlortetracycline does not occur when used in combination with previously declared safe individual agents. The agent is obviously present in its active form when the assay method is critical enough to determine its presence. Generally, agents are specifically different in their molecular structures and actions so that interference by following similar metabolic pathways within the body is unlikely, and too, the agent in numerous cases is absorbed and then eliminated as the intact or slightly modified molecule.

At the present time, compatibility of agents within a combination must be proved by two separate experiments:

The effects on absorption, excretion, and tissue residues are determined by assays of blood and tissues from animals given each specific agent alone and animals given the combination in question.

The effect on efficacy is determined by comparing the results of treating animals experimentally infected with specific diseases with the agent alone or with the combination in question.

When the results of these two tests are the same for the combination groups as for those treated with the one agent, it is concluded that they are completely compatible. To facilitate the clearance of more combinations, it seems that the induced disease experiment could be safely eliminated for those agents requiring microbiological assay procedures, and even for those agents that are determined by chemical procedures where their presence alone has previously shown them to control a condition effectively. In the case of antibiotics, if equal blood levels are found, the antibacterial activity has not been affected by the combination since blood levels are determined by a microbiological method. Thus, there is little reason to believe that other sensitive organisms, such as those that would be used in an induced disease test, would respond differently.

These statements are based on the observation that blood and tissue levels are the same when the agent is used alone as when the combination of agents is used. Under these circumstances, only blood level data should be required.

It may be argued further that when the blood levels of the combination-treated groups are the same as the blood levels of the single agent-treated group, it becomes evident that the combination of agents did not destroy the single agent while in the feed or in the gut of the animal; the agent's absorption or excretion was not changed by the presence of the other members of the combination; the single agent has not been altered in its metabolism by the combination; and the tissue residues have not been increased by the combination.

Literature Cited

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FEED ADDITIVES

The Impact on the Analytical Chemist of Government Regulations Pertaining to Tissue Residues

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CHEMICAL SUBSTANCES added to animal feeds and fed to food-producing animals may produce residues of the substance in the animal tissue by indirect means. These residues are regarded as food additives and are regulated accordingly under Section 409 of the Federal Food, Drug, and Cosmetic Act. Pesticide chemicals may be used on raw agricultural commodities and may also cause residues in or on the plant tissue. These residues are regulated accordingly under Section 408 of the Act. It is this type of indirect additive, or residue, which will be discussed in this paper.

The problems associated with directing analytical work in measuring residues become extremely complex in view of current regulations and the interpretation of these regulations. This paper will discuss some of these complexities and will suggest possible solutions to some of the problems involved in complying with the regulations. It is hoped that through continued discussion of the many ramifications of the regulations that industry and the FDA will eventually agree on reasonable solutions to their mutual problems.

Problems Associated with "Zero" Residue

It is generally agreed that absolute zero is impossible to attain with present analytical methodology, since it would be necessary to detect one molecule of substance per some quantity of tissue. Residues must therefore be considered in terms of "relative zero." FDA regards relative zero as the level represented