I. GENERAL INFORMATION

A. File Number

NADA 138-703

B. Sponsor

A. L. Laboratories, Inc.

C. Proprietary Name

Albac®, 3-Nitro® and Coban®

D. Established Name

bacitracin zinc, roxarsone, and monensin sodium

E. Dosage Form

Medicated finished feeds manufactured from approved separate feed additive premixes for broiler chickens. Albac®, each pound of premix contains 50 grams bacitracin activity. 3-Nitro® , each pound of premix contains either 45.5, 90.9, 227.3 or 363.6 grams roxarsone. Coban®, each pound of premix contains 45 grams monensin as monensin sodium.

F. Dispensing Status

OTC

G. Dosage Regimen

Bacitracin Zinc: 4-50 grams per ton
Roxarsone: 22.7-45.4 grams per ton (0.0025%-0.005%)
Monensin Sodium: 90-110 grams per ton

H. Route of Administration

For oral administration *via* the feed for growing broiler chickens.

I. Species/Class

Species and class (if applicable)

J. Indication

For improved feed efficiency and as an aid in prevention of coccidiosis caused by *Eimeria necatrix, E. tenella, E. acervulina, E. brunetti, E. maxima,* and *E. mivati.*

K. Effect of Supplement

This supplement provides for <changes being approved> (Delete this section for Original approvals)

II. EFFECTIVENESS

A. Floor Pen Studies

Three floor pen experiments were conducted using approved protocols and 5,640 broiler chickens. Bacitracin zinc was used at 0 or 50 g/ton of feed in combination with roxarsone at 0 or 45.4 g/ton of feed to provide all 4 possible combinations of these 2 drugs. Monensin sodium at 110 g/ton was included in all experimental diets. The experiments were designed to test the effects of bacitracin zinc and roxarsone in combination with monensin on body weight gains and feed efficiency. A description of the three experiments follows:

1. Experiment No. TX-B-112-83

   Investigator:

   William F. Krueger, Ph.D.
   Department of Poultry Science
   Texas A&M University
   College Station, TX 77843

   Monitor:

   Ralph V. Fell, Ph.D.
   Route 9, Box 42
   Pine Bluff, AR 71603

   This 47-day experiment was conducted in a conventional type broiler house at the poultry research facilities, Texas A&M University, College Station, TX from January 6, 1984 to February 15, 1984. The experiment consisted of four dietary treatments which were monensin at 110 g/ton, monensin at 110 g/ton plus bacitracin zinc at 50 g/ton, monensin at 110 g/ton plus roxarsone at 45.4 g/ton and monensin at 110 g/ton plus bacitracin zinc at 50 g/ton plus roxarsone at 45.4 g/ton. Three thousand day-old, sexed, Cobb x Hubbard Commercial broiler chicks from one hatchery supply flock were placed randomly by sex in 30 pens to provide 50 male and 50 female chicks per pen. Treatments were randomly assigned to blocks of pens. Each 8' x 10' pen was identically equipped with a six foot mechanical waterer and hand feeders. The pens had dirt floors covered with used litter and topped with an inch layer of fresh pine shavings. Each pen had its own artificial light source of a 40 watt light bulb. Artificial light was provided for 22 hours of total light per day.

2. Experiment No. MO-B-114-84

   Investigator:

   Randall A. Primo
   Ponderosa Research Company
   French Village, MO 63036

   Monitor:

   Ralph V. Fell, Ph.D.

Route 9, Box 42
Pine Bluff, AR 71603

This 48-day experiment was conducted from December 16, 1983 to February 2, 1984 in a conventional, insulated, curtain-sided broiler house with poultry wire partitions and dirt floors at the Ponderosa Research Company facilities. The experiment consisted of four dietary treatments which were monensin at 110 g/ton, monensin at 110 g/ton plus bacitracin zinc at 50 g/ton, monensin at 110 g/ton plus roxarsone at 45.4 g/ton and monensin at 110 g/ton plus bacitracin zinc at 50 g/ton plus roxarsone at 45.4 g/ton. Twelve hundred day-old, sexed broiler chicks were placed randomly by sex in 24 pens to provide 25 male and 25 female chicks per pen. Treatments were randomly assigned to blocks of pens. The four dietary treatments were then randomly assigned. Each 5'x 8' pen was identically equipped with a Mono-Flo automatic water fountain, a cylindrical tube-type hanging self feeder and a thermostatically controlled heat lamp brooder. Natural light was supplement by a 25 watt bulb over each pen.

3. Experiment ARK-B-120-84

   Investigator:

   Park W. Waldroup, Ph.D.
   Department of Animal Sciences
   University of Arkansas
   Fayetteville, AR 72701

   Monitor:

   Ralph V. Fell, Ph.D.
   Route 9, Box 42
   Pine Bluff, AR 71603

   This 49-day experiment was conducted in a conventional steel-truss building with insulated roof and side walls, a 3 foot curtain and automatic fans at the poultry research facilities, University of Arkansas, Fayetteville, AR from April 2, 1984 to May 21, 1984. The experiment consisted of four dietary treatments which were monensin at 110 g/ton, monensin at 110 g/ton plus bacitracin zinc at 50 g/ton, monensin at 110 g/ton plus roxarsone at 45.4 g/ton and monensin at 110 g/ton plus bacitracin zinc at 50 g/ton plus roxarsone at 45.4 g/ton. One thousand four hundred and forty day-old, sexed broiler chickens from a commercial hatchery were placed randomly by sex in 24 pens to provide 30 male and 30 female chicks per pen. Treatments were randomly assigned to blocks of pens. Each 7' x 8' pen was equipped with two hanging tube-type feeders, an automatic water fount and an infrared brooder. During the first 7 days supplemental feed and water were available.

4. Summary of Floor-Pen Studies

   The three described floor-pen studies, using approved protocols and 5,640 broiler chickens (equal numbers of males and females), were conducted under conditions simulating actual field use to determine the growth promoting effects

of bacitracin zinc and roxarsone in combination with monensin sodium. The studies were conducted in three different geographical locations.

Consistent with pen size and allowing 0.8 to 0.9 square feet per bird, 50 to 100 birds of equal sex were selected at random and assigned to pens. Six or seven replicates were used per treatment group. Bacitracin zinc at 0 and 50 grams per ton of feed was used in combination with roxarsone at 0 or 45.4 g/ton of feed to provide all 4 of the possible combinations of these drugs. Monensin sodium at 110 grams per ton of feed was used in all feed in each study. The studies were designed and conducted to simulate varying conditions as climate, geographical location, weather, management practices, and degree of disease contamination of the premises.

Feed consumption was corrected for mortality by estimating feed consumed from standard tables based on age of each bird when it died in each pen and subtracting that amount from the total feed consumed by that pen.

A detailed statistical evaluation of the three studies was conducted by Dr. Charles Gates of the Institute of Statistics, Texas A&M University, College Station, TX. The data were combined over the trials and analyzed using SAS's GLM Procedures (*). A combined analysis of variance showed that the bacitracin zinc significantly improved feed efficiency ($P < 0.01$) independent of the dose of roxarsone (Table 1). In addition, roxarsone significantly improved feed efficiency ($P < 0.05$) independent of the dose of bacitracin zinc.

Based on the results of specific treatment comparisons of data from the three floor pen studies, this application qualifies for full range approval for the three-way combination of bacitracin zinc, roxarsone and monensin under CVM policy for combination drugs.

Based on the revised Drug Combination Guidelines, the data adequately demonstrate the contribution of both bacitracin zinc and roxarsone on the feed efficiency of broiler chickens and support the use of 4-50 grams bacitracin zinc and 22.7-45.4 grams roxarsone per ton of feed for improved feed efficiency in the presence of 90-110 grams monensin sodium per ton.

The claim for the combination is: "As an aid in the prevention of coccidiosis caused by Eimeria necatrix, E. tenella, E. acervulina, E. brunetti, E. maxima and E. mivati; and for improved feed efficiency."

* (SAS User's Guide: Statistics, 1982 Edition, SAS Institute, Cary, NC)

TABLE 1 Summary of Response of Broilers to Bacitracin Zinc and/or Roxarsone When Fed With Monensin

		Monensin, 110 g/ton		Monensin, 110 g/ton plus BZN, 50g/ton		Monensin, 110 g/ton plus Roxarsone, 45.4 g/ton		Monensin, 100g/ton plus BZN, 50 g/ton plus Roxarsone, 45.4 g/ton	
	No. Reps	**Average Weight**	**Feed/ Gain**	**Average Weight**	**Feed/ Gain**	**Average Weight**	**Feed/ Gain**	**Average Weight**	**Feed/ Gain**
TX-B-112-83	7	3.62	2.26	3.77	2.11	3.88	2.10	3.79	2.11
MO-B-I14-84	6	4.18	2.26	4.26	2.20	4.12	2.25	4.27	2.18
ARK-B-120-84	5	4.33	2.10	4.52	2.03	4.52	2.05	4.54	1.99
Average		4.04	2.20	4.18	2.11	4.17	2.13	4.20	2.09

B. Effectiveness - Non-interference Studies:

Two adequate and well controlled battery studies, using approved protocols and 400 Arbor Acres/Peterson broiler chickens, equal numbers of males and females, were conducted in environmentally controlled animal rooms with even lighting and power ventilation, located at the experimental poultry farm of the University of Georgia, Department of Poultry Science, near Athens, GA.

1. Experiment No. GA-B-118-85; GA-B-118-85/2

   Investigator:

   Larry R. McDougald, Ph.D.
   Department of Poultry Science
   University of Georgia
   Athens, GA 30602

   Monitor:

   Ralph V. Fell, Ph.D.
   Route 9, Box 42
   Pine Bluff, AR 71603

   Each of eight treatments (see Table 2) were replicated with 4 cages of 10 wing banded birds each (5 males and 5 females) in the two separate studies. The birds were given unmedicated broiler starter until they were 12 days old, then they were weighed, allocated to cages according to weight by means of a Statistical Analysis System Program, and given medicated feed. At 14 days of age, the birds were reweighed and infected with mixed cultures of coccidia. The infective cultures were E. mitis, E. necatrix and E. brunetti in Study Ia; and E. acervulina, E. maxima and E. tenella in Studies Ib and 2. The coccidia species that were used were recent isolates obtained from commercial poultry farms in the United States.

   Data, including death losses, intestinal lesion scores, droppings scores, weight gain and feed consumption were recorded over the 2 weeks following infection (Table 2). The infections reduced weight gains in the infected controls, compared with the uninfected controls. Treatment with monensin was effective in improving weight gain in all instances, whether it was used alone or in combination with bacitracin zinc and/or roxarsone. Lesion scores were recorded for the upper, mid

and cecal areas of the gut. Monensin was effective in controlling coccidiosis caused by the six species of Eimeria in the broiler chickens. The use of bacitracin zinc and roxarsone with monensin did not interfere with the anti-coccidial action of monensin.

TABLE 2 Anti-coccidial Activity of Monensin in Combination with Bacitracin Zinc and Roxarsone Against Mixed Eimeria Infections in Young Broiler Chickens

Medicated	g/ ton	Average Live Weight (g)			Mortality			Total Lesion Scores/Bird			Total Droppings Scores/Bird		
		Study 1a	Study 1b	Study 2	Study 1a	Study 1b	Study 2	Study 1a	Study 1b	Study 2	Study 1a	Study 1b	Study 2
None, uninfected	0	461.4	441.7	454.9	0/40	0/40	0/40	0	0	0	0	0	0
None	0	357.1	138.2	127.0	1/40	33/40	27/40	4.75	12.00	12.00	7.25	8.25	15.50
Monensin	90	389.7	303.4	412.0	0/40	6/40	2/40	0.69	5.50	5.30	0.75	4.00	9.25
Bacitracin Zinc	100	256.9	-	100.8	2/40	-	27/40	5.88	-	12.00	5.00	-	15.75
Monensin + Bacitracin Zinc	90 100	358.6	-	413.4	0/40	-	2/40	0.88	-	6.38	0.75	-	9.25
Bacitracin Zinc + Roxarsone	100 45	145.1	242.1	-	0/40	21/40	-	6.38	12.00	-	5.00	6.75	-
Monensin + Bacitracin Zinc + Roxarsone	90 100 45	368.7	327.9	-	0/40	5/40	-	0.56	6.25	-	0	2.00	-
Monensin + Roxarsone	90 45	427.8	399.7	-	0/40	11/40	-	0.69	6.50	-	0.25	2.00	-

Study 1a, infective cultures were from field isolates of *E. mitis*, *E. necatrix* and *E. brunetti* (Study GA-B-118-85). Study 1b, infective cultures were from field isolates of *E. acervulina*, *E. maxima* and *E. tenella* (Study GA-B-l18-85). Study 2, infective cultures were from field isolates of *E. acervulina*, *E. maxima* and *E. tenella* (Study GA-B-I 18-85/2).

III. TARGET ANIMAL SAFETY

The basic animal safety data for the individual drugs may be found in the original NADA 098-452 for bacitracin zinc, NADA 038-878 for monensin and NADA 007-891 for 3-Nitro (also MF 19 for roxarsone). The effectiveness studies shown in Section IV demonstrate that no ill effects occurred when the drugs were combined indicating that they are as safe when fed in combination as when fed alone.

This application is in accord with the Animal Safety Guidelines. Additional safety studies were not required because: (1) The drugs have been approved singly and (2) adequate documentation has been provided to show that these compounds are compatible in combination when used in broiler chicken feeds. Therefore, based on the data in the original NADAs, the non-interference study, the floor-pen efficacy studies, and the drug residue elimination study, it is concluded that this combination of drugs may be safely fed to broiler chickens.

IV. HUMAN FOOD SAFETY

It has been demonstrated in the original NADAs (098-452 for bacitracin zinc, 038-878 for monensin and 007-891 for 3-Nitro) and in MF 19 for roxarsone that these products are not a hazard to human health when used according to approved labeling. Tolerances for residues of bacitracin zinc are established at 0.5 ppm, negligible residue, in uncooked tissue of chickens (21 CFR 556.70). Tolerances for arsenic in the edible tissues of chickens are established at 0.5 ppm in muscle and 2 ppm in edible by-products (21 CFR 556.60). Tolerances for residues of monensin in edible tissues of chickens are 0.05 ppm (21 CFR 556.420). The residue data supporting the individual uses of bacitracin zinc, roxarsone and monensin have been presented in the original applications for each.

A. Tissue Residue Studies

In the first tissue residue study (AEF-1-83) with monensin, roxarsone and bacitracin zinc, the recovery of bacitracin from spiked control tissue was below the acceptable level. A second tissue residue study (MO-B-TR-1-85) was conducted to test for bacitracin residues.

	AEF-1-83 (Monensin, Roxarsone)	**MO-B-TR-1-85 (Bacitracin Zinc)**
Investigator	Thomas Kennedy, Ph.D. AEF Research, Inc. Waunakee, WI 53597	Randall A. Primo Ponderosa Research Company French Village, MO 63036
Monitor	Cornell Johnson 5133 Caton Lane Waunakee, WI 53597	Ralph V. Fell, Ph.D. Route 9, Box 42 Pine Bluff, AR 71603

Both residue studies were conducted in a similar manner. Commercial broiler chicks were divided into two groups containing equal numbers of each sex. One group was maintained on a non-medicated broiler feed to provide control tissues for drug recovery and non-interference studies. The other group was fed the same basal feed medicated with monensin at 110 g/ton, roxarsone at 45.4 g/ton and bacitracin zinc at 100 g/ton. Birds remained on test until at least 48 days of age after which some birds were withdrawn from medicated diet for 0, 1, 2, 3 or 5 days. The weight gains and feed efficiencies of the birds in both studies were similar to the weight gains and

feed efficiencies obtained in the broiler industry. Birds of each sex were randomly selected and sacrificed for each drug to be assayed and for each withdrawal time. Chickens were processed in a manner similar to commercial practices. Tissue samples were removed from each bird and placed in individual plastic tissue bags and labeled for treatment, chicken number, sex, tissue and days withdrawn from drugs. All tissues were quick frozen and shipped on dry ice to the analytical laboratories for drug assay.

The summary of the tissue residue depletion studies are shown in Table 3. These data establish that each drug in the presence of the other two drugs does not exceed its established safe concentration or tolerance.

Tissue assay non-interference and method validation studies for bacitracin zinc tissue assay were conducted by spiking control samples with bacitracin zinc, roxarsone and monensin and then assaying for bacitracin residues. The assay results demonstrated no interference by monensin and roxarsone for bacitracin (MO-B-TR-1).

A non-interference study for monensin was conducted by spiking control tissue samples with monensin, roxarsone and bacitracin zinc and then assaying these tissues for monensin content. The results demonstrated no interference by bacitracin zinc and roxarsone for monensin.

Assay non-interference data are not required for roxarsone due to the procedure for arsenic analysis.

TABLE 3 Residue Depletion Assay Results

		Tissue			
		Liver	**Kidney**	**Skin/Fat**	**Muscle**
Monensin (AEF-1-83)	**Established safe concentration, ppm**	--	--	0.05	--
	Monensin residue: 2-day withdrawal	No detectable residue	No detectable residue	No detectable residue	No detectable residue
	Monensin residue: 3-day withdrawal	No detectable residue	No detectable residue	No detectable residue	No detectable residue
Bacitracin Zinc (MO-B-TR-1-85)	**Established tolerance, ppm**	--	--	--	0.5
	Bacitracin residue: 0-day withdrawal	No detectable residue	No detectable residue	No detectable residue	No detectable residue
	Bacitracin residue: 1-day withdrawal	No detectable residue	No detectable residue	No detectable residue	No detectable residue
Roxarsone (AEF-1-83)	**Established tolerance, ppm**	2.0	2.0	2.0	0.5
	Roxarsone residue: 0-day withdrawal	1.0	0.7	0.3	0.2
	Roxarsone residue: 3-day withdrawal	0.5	0.3	0.1	0.1
	Roxarsone residue: 5-day withdrawal	0.4	0.3	0.1	0.1

Monensin Assay Procedure No. 5801450 for chicken tissues and eggs. Eli Lilly and Company, Greenfield Laboratories, Greenfield, IN.

Bacitracin Assay Procedure from Antibiotic Residues in Milk, Dairy Products and Animal Tissues, Methods, Reports and Protocols, Rev. October 1968. National Center for Antibiotic and Insulin Analysis. Food and Drug Administration, Department of Health, Education and Welfare, Washington, DC 20204.
Arsenic Assay Procedure No. 5801380 for chicken tissues essentially as described in Official Methods of Analyses of Association of Official Analytical Chemists, 11th Edition, p 402 (1970).

Based upon the established withdrawal times for monensin, bacitracin zinc and roxarsone, no residues were above tolerances in any tissues from these broiler chickens. The data support a 5-day withdrawal period for the bacitracin zinc, monensin, roxarsone combination because of the required 5-day withdrawal period for roxarsone.

V. AGENCY CONCLUSIONS

The data submitted in support of this NADA comply with the requirements of Section 512 of the Act and demonstrate that monensin (90-110 g/ton) plus bacitracin zinc (4-50 g/ton) plus roxarsone (22.7-45.4 g/ton) are safe and effective for the claims indicated in Section II of this FOI summary.

This original NADA is regarded as a Category II application under CVM's supplemental policy (42 FR 64367) which did not require reevaluation of safety and efficacy data in the parent NADAs. The drugs are to be supplied to the feed mill in individual premixes for combining in finished feeds (Type C Article) within the indications and dosages approved in the parent NADAs.

Residue depletion studies demonstrate that monensin, bacitracin zinc and roxarsone deplete to levels well below tolerance levels within the required five days withdrawal time before the birds are slaughtered. See Table 3 for residue depletion assay results and tolerance levels.

Non interference studies demonstrated that monensin prevented an outbreak of coccidiosis alone and in the presence of bacitracin zinc and roxarsone when the birds were exposed to the six major species of Eimeria. The data from three well controlled floor pen studies demonstrate the effectiveness of bacitracin zinc (50 g/ton) .and of roxarsone (45.4 g/ton) in the presence of monensin (110 g/ton). These data qualify the application for approval under CVM's policy outlined in the combination drug guidelines revised October 1983. This policy permits the granting of range approval for bacitracin zinc (4-50 g/ton) plus roxarsone (22.7-45.4 g/ton) plus monensin (90-110 g/ton) as an aid in the prevention of coccidiosis and for improved feed efficiency in broiler chickens as shown in Section II of this FOI Summary.

VI. ATTACHMENTS

Copies of applicable labels may be obtained by writing to the:

Food and Drug Administration
Freedom of Information Staff (HFI-35)

5600 Fishers Lane
Rockville, MD 20857

Or requests may be sent via fax to: (301) 443-1726. If there are problems sending a fax, call (301) 443-2414.

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.