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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Stability of Bacitracin Methylene Disalicylate in the Presence of Pellet Binding Agents in Poultry Feed

P. W. Waldroup[1], S. E. Watkins[1], J. T. Skinner[2] and A. D. Desai[2]
[1] Poultry Science Department, University of Arkansas, Fayetteville AR 72701, USA
[2]Alpharma Inc., One Executive Drive, Fort Lee, NJ 07024, USA
Waldroup@uark.edu

Abstract: A study was conducted with broiler feed medicated with bacitracin methylene disalicylate (BMD) to determine if the addition of lignin sulfonate pellet-binding agents affects the assay of active concentration of BMD in pelleted feed. This study also examined the stability of BMD in the presence of these pellet-binding agents in pelleted feed over extended storage. Diets were mixed that contained 27.5 mg/kg of BMD. Diet 1 did not contain a pellet binder. Diet 2 contained 1.25% Ameri-Bond 2x, and Diet 3 contained 0.6% Pel-Stik. Mash and pelleted samples of each diet were assayed for loss on drying (LOD) and BMD level during week 0. Pelleted samples were stored at ambient temperature (25° C) and ambient humidity, or elevated temperature (37° C) and ambient humidity. Samples stored at 25° C were assayed during weeks 2, 4, 7, 10, and 13, and those stored at 37° C were assayed during weeks 1, 2, 3, and 4 for LOD and BMD levels. Assay results from week 0 in both mash and pelleted feeds demonstrated the non-interference of the pellet binders with the assay of BMD. The BMD assay results of feeds stored at 25 and 37° C demonstrated that these pellet binders do not affect the stability of BMD in feed.

Key words: Antibiotics, stability, pellet binders, interference

Introduction

Pellet binders improve the quality of pelleted feeds by means of increased bulk density, improved flow characteristics, and decreased dustiness. Feeds are more effectively delivered to the animals when pellet quality is high. In order to provide high pellet quality, non nutritive agents known as pellet binders are often added to the feed. However, some pellet binders have been shown to interfere with nutrient absorption (Briggs and Fox, 1956; Sibbald and Wolynetz, 1989).

To meet regulatory requirements, it is essential that persons using antibiotics in animal feeds periodically monitor dietary levels to insure compliance. Because most broiler diets are fed in pelleted form, the objective of the present study was to determine the effects of the pelleting process in the presence of pellet-binding agents or on the active concentration of bacitracin methylene disalicylate (BMD) in feed, and to determine the stability of BMD in the presence of pellet-binding agents in pelleted feed.

Materials and Methods

A diet was formulated that met the nutritional requirements for the growing broiler (3 to 6 weeks of age) as suggested by the NRC (1994). Grower feed was chosen because it is commonly fed in a pelleted form. The composition of the feed is shown in Table 1. Three 1000-pound (454 kg) batches of feed were prepared. Approximately equal amounts of each basal were used to prepare the experimental diets. Each experimental diet contained 27.5 mg/kg BMD (Alpharma Inc., Fort Lee NJ 07024). Diet 1 did not contain a pellet binder. Diet 2 contained 1.25% Ameri-Bond 2x (Ligno Tech USA, Inc., Greenwich CT 06830) as the pellet binder, and Diet 3 contained 0.6% Pel-Stik (Hess & Clark, Inc., Ashland OH 44805) as the pellet binder. Ameri-Bond 2x is described by the manufacturer as a modified calcium lignosulfonate product. The manufacturer describes Pel-Stik as a mixture of dehydrated lignin sulfonate, propylene glycol, hydrolyzed animal bone collagen, sodium acetate, and sodium sulfate. The levels of the pellet binders used in this experiment are higher than those normally used by feed manufacturers in North America to provide a worse case scenario for testing the drug.

Mash and pelleted samples of each feed were taken. Pelleting was accomplished in a 30 hp pellet mill (Master Model Pellet Mill, California Pellet Mill Company, Crawfordsville IN) with steam conditioning. Pelleting temperature for each mix was 84° C. Upon completion of mixing, pelleting and sampling of the various diets, one mash and one pelleted sample of each experimental diet were shipped overnight to a testing laboratory (Alpharma, Inc., Chicago Heights IL 60411).

Samples of mash and pelleted feeds were assayed for loss on drying (LOD) and bacitracin (BMD) activity upon arrival (0 weeks) at the laboratory. Samples of mash feed were assayed during week 0 only. Samples of pelleted feeds were stored in environments with ambient temperature (25° C) and ambient humidity, or with elevated temperatures (37° C) and ambient humidity. Temperatures in each environment were recorded daily. Samples stored at 25° C were assayed for LOD and BMD during weeks 0, 2, 4, 7, 10, and 13, and those stored at 37° C were assayed during weeks 0, 1, 2, 3, and 4. All assays (BMD and LOD) were conducted in triplicate with the exception of pelleted feeds

Table 1: Composition of broiler diet used in study

Ingredient	g/kg
Yellow corn	681.80
Soybean meal 48%	172.20
Poultry byproduct meal	50.00
Blended animal protein[1]	50.00
Poultry oil	26.34
Ground limestone	10.34
Vitamin premix[2]	5.00
Feed grade salt	2.08
Dicalcium phosphate	1.22
Trace mineral mix[3]	1.00
DL-Methionine (98%)	0.02
TOTAL	1000.00

[1]Pro-Pak, H.J. Baker & Bro., Stamford, CT.
[2]Provides per kg of diet: vitamin A (from vitamin A acetate) 7714 IU; cholecalciferol 2204 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg.
[3]Provides per kg of diet: Mn (from $MnSO_4 \cdot H_2O$) 100 mg; Zn (from $ZnSO_4 \cdot 7H_2O$) 100 mg; Fe (from $FeSO_4 \cdot 7H_2O$) 50 mg; Cu (from $CuSO_4 \cdot 5H_2O$) 10 mg; I from $Ca(IO_3)_2 \cdot H_2O$), 1 mg.

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Table 2: Effect of pelleting a broiler grower feed in the presence of pellet binders on the initial concentration of bacitracin methylene disalicylate (BMD)

Measurement	Type of pellet binder		
	None	Ameri-Bond 2x[1]	Pel-Stik[2]
A. Mash diets			
BMD, mg/kg recovered[3]	28.5 ± 2.0	28.9 ± 1.3	28.9 ± 1.5
BMD, % recovered[4]	103.5 ± 7.3	104.9 ± 4.6	105.2 ± 5.3
Loss on drying (LOD) %	12.0 ± 0.3	11.8 ± 0.5	11.7 ± 0.1
BMD, mg/kg adjusted for LOD	32.3 ± 2.4	32.7 ± 1.3	32.8 ± 1.6
BMD, % adjusted for LOD	117.6 ± 8.7	119.0 ± 4.6	119.2 ± 5.9
B. Pelleted dietsBMD,			
mg/kg recovered	26.5 ± 1.3	26.9 ± 1.4	25.1 ± 1.5
BMD, % recovered	96.2 ± 4.6	97.8 ± 5.0	91.2 ± 5.5
Loss on drying (LOD) %	13.1	12.0	11.9
BMD, mg/kg adjusted for LOD	30.5 ± 1.4	30.6 ± 1.6	28.5 ± 1.7
BMD, % adjusted for LOD	110.8 ± 5.2	111.2 ± 5.7	103.5 ± 6.2

[1]LignoTech USA,Inc.,Greenwich CT 06830. [2]Hess & Clark, Inc., Ashland OH 44805. [3]All feeds were prepared to contain 27.5 mg/kg of BMD. [4]Percentage of claimed amount of BMD recovered.

Table 3. Effects of pellet binders on the stability of bacitracin methylene disalicylate (BMD) in feeds stored at 25° C over 13 weeks

Measurement	Pellet binder	Weeks of storage at 25°C					
		0	2	4	7	10	13
BMD, mg/kg recovered[1]	None	26.5 ± 1.3	25.3 ± 0.4	22.2 ± 0	24.0 ± 4.1	22.4 ± 2.5	26.0 ± 1.5
	Ameri-Bond 2x[2]	26.9 ± 1.4	24.7 ± 1.3	21.9 ± 0.7	25.3 ± 0.7	23.9 ± 1.5	26.1 ± 1.4
	Pel-Stik[3]	25.1 ± 1.5	24.4 ± 1.7	22.4 ± 2.9	21.4 ± 1.3	22.7 ± 1.7	26.7 ± 1.6
BMD, % recovered[4]	None	96.2 ± 4.6	92.0 ± 1.6	80.7 ± 0.0	87.4 ± 14.9	81.3 ± 9.1	94.7 ± 5.5
	Ameri-Bond 2x	97.8 ± 5.0	89.8 ± 4.9	79.6 ± 2.4	91.9 ± 2.4	86.9 ± 5.4	94.9 ± 5.0
	Pel-Stik	91.1 ± 5.5	88.7 ± 6.1	81.33 ± 10.5	77.8 ± 4.8	82.7 ± 6.1	96.9 ± 5.8
Loss on drying (LOD) %	None	13.1	11.1 ± 0.2	10.4 ± 0.1	8.3 ± 0.3	7.6 ± 0.5	7.3 ± 0.6
	Ameri-Bond 2x	12.0	11.0 ± 0.6	9.9 ± 0.5	8.3 ± 0.4	7.8 ± 0.1	7.1 ± 0.5
	Pel-Stik	11.9	11.5 ± 0.4	10.0 ± 0.5	8.5 ± 0.3	8.3 ± 0.3	7.3 ± 0.6
BMD, mg/kg adjusted for LOD	None	30.5 ± 1.4	28.4 ± 0.6	24.8 ± 0.0	26.2 ± 4.6	24.2 ± 2.8	28.1 ± 1.5
	Ameri-Bond 2x	30.6 ± 1.6	27.8 ± 1.6	24.3 ± 0.6	27.6 ± 0.6	25.9 ± 1.6	28.1 ± 1.5
	Pel-Stik	28.5 ± 1.7	27.6 ± 1.8	24.9 ± 3.3	23.4 ± 1.5	24.8 ± 1.8	28.8 ± 1.7
BMD, % adjusted for LOD	None	110.7 ± 5.2	103.4 ± 2.0	90.1 ± 0.1	95.4 ± 15.6	88.0 ± 10.1	102.1 ± 5.4
	Ameri-Bond 2x	111.2 ± 5.7	100.9 ± 5.7	88.4 ± 2.3	100.2 ± 2.3	94.3 ± 2.3	102.1 ± 5.4
	Pel-Stik	103.5 ± 6.2	100.3 ± 6.7	90.4 ± 12.1	85.1 ± 5.5	90.2 ± 6.5	104.6 ± 6.3

[1]All feeds were prepared to contain 27.5 mg/kg of BMD. [2]LignoTech USA,Inc.,Greenwich CT 06830. 3Hess & Clark, Inc., Ashland OH 44805. [4]Percentage of claimed amount of BMD recovered.

during week 0, where only a single assay of each feed was conducted for LOD. Bacitracin activity was measured using a variant strain of *Micrococcus luteus* ATCC 10240 (Mathers and Desai, 1995).

The percentage of BMD recovered was calculated by dividing the amount of BMD assayed for a sample by the claimed amount and then multiplied by 100. The amount and percentage of BMD recovered was adjusted to a dry matter basis by dividing each by the dry matter content [(100-LOD)/100] of the sample. Summary statistics were generated by using the PROC MEANS procedure of SAS (SAS Institute, 1988). The PROC REG procedure was used to fit a linear line to the data.

Results and Discussion

BMD assay results from week 0 for both mash and pelleted feeds demonstrate the non-interference of Ameri-Bond 2x and Pel-Stik with the assay of BMD (Table 2). Mean BMD assays for all these feeds were within the claimed level (assay limits for BMD in finished feeds are ±25% of the claim in Canada and ±30% of the claim in the United States). During week 0, the amount of BMD recovered from the mash feeds was approximately 100% of the claimed level, and the amount of BMD recovered from the pelleted feeds was greater than 90% of claim.

The percentage of LOD from feeds decreased over time, so the BMD assay results are presented on both an as is and a dry matter basis. This reduction in the percentage of LOD over time was significant ($P = 0.0001$) for samples stored in both environments (25° C with ambient humidity and 37° C with ambient humidity). BMD assay results of feeds stored at 25° C over the 13 week period (Table 3) demonstrate that the presence of these pellet binders in feeds do not affect the stability of BMD. Most poultry feeds in the United States and Canada are consumed by the animals within four weeks of manufacturing. There was some fluctuation in the levels of BMD recovered from time period to time period, but this fluctuation did not appear related to the presence or absence of the pellet binders but rather to assay variation. The linear regression model did not fit the data.

BMD assay results of feeds stored at 37° C over the 4 week period (Table 4) demonstrate that the presence of these pellet binders in feeds do not affect the stability of BMD. In this environment, the amount of BMD recovered tended to decrease with time but this decrease in activity did not appear related to the presence or absence of the pellet binders. The linear regression model did not fit the data well (best $R^2 = 0.57$); however, there were significant ($P < .05$) linear reductions in the amount of BMD recovered over the 4 week period from feed containing BMD alone (with or without the adjustment for LOD) and those containing Ameri-Bond 2x (adjusted for LOD). Even after 4 weeks of storage in this extreme environment, the amounts of BMD recovered from these feeds (with or without pellet binders) were within its allowable assay limits.

Although feed manufacturers recognize that proper mixing of feed ingredients is important, comparatively few trials address the

Table 4: Effects of pellet binders on the stability of bacitracin methylene disalicylate (BMD) in feeds stored at 37°C over four weeks

Measurement	Pellet binder	Weeks of storage at 37°C				
		0	1	2	3	4
BMD, mg/kg recovered[1]	None	26.5 ± 1.3	28.4 ± 0.6	22.9 ± 1.8	24.0 ± 1.7	22.7 ± 1.5
	Ameri-Bond 2x[2]	26.9 ± 1.4	29.6 ± 0.6	25.5 ± 2.6	26.6 ± 2.1	25.6 ± 2.2
	Pel-Stik[3]	25.1 ± 1.5	27.9 ± 1.8	27.0 ± 2.0	25.1 ± 1.5	25.7 ± 0.7
BMD, % recovered[4]	None	96.2 ± 4.6	103.3 ± 2.0	83.4 ± 6.4	87.3 ± 6.4	82.4 ± 5.5
	Ameri-Bond 2x	97.8 ± 5.0	107.8 ± 2.1	92.7 ± 9.3	96.7 ± 7.6	92.9 ± 7.8
	Pel-Stik	91.1 ± 5.5	101.5 ± 6.4	98.3 ± 7.2	91.3 ± 5.4	93.6 ± 2.6
Loss on drying (LOD) %	None	13.1	9.7 ± 0.5	7.2 ± 0.4	8.7 ± 0.4	7.5 ± 0.5
	Ameri-Bond 2x	12.0	8.7 ± 0.9	7.4 ± 0.6	8.8 ± 0.5	7.1 ± 0.2
	Pel-Stik	11.9	8.6 ± 0.2	7.5 ± 0.8	8.3 ± 0.4	6.9 ± 0.1
BMD, mg/kg adjusted for LOD	None	30.5 ± 1.4	31.4 ± 0.5	24.7 ± 1.9	26.3 ± 1.9	24.5 ± 1.7
	Ameri-Bond 2x	30.6 ± 1.6	32.5 ± 0.3	27.5 ± 2.8	29.2 ± 2.4	27.5 ± 2.4
	Pel-Stik	28.5 ± 1.7	30.5 ± 1.9	29.2 ± 2.1	27.4 ± 1.7	27.7 ± 0.8
BMD, % adjusted for LOD	None	110.7 ± 5.2	114.4 ± 1.9	89.9 ± 6.8	95.6 ± 6.9	89.1 ± 6.2
	Ameri-Bond 2x	111.2 ± 5.7	118.1 ± 1.2	100.16 ± 10.3	106.1 ± 8.8	100.05 ± 8.7
	Pel-Stik	103.5 ± 6.1	111.0 ± 7.0	106.3 ± 7.8	99.6 ± 6.1	100.6 ± 2.8

[1]All feeds were prepared to contain 27.5 mg/kg of BMD. [2]LignoTech USA,Inc.,Greenwich CT 06830. 3Hess & Clark, Inc., Ashland OH 44805. [4]Percentage of claimed amount of BMD recovered.

problems of stability of feed additives following pelleting or storage. Studies have addressed the problems of maintaining stability of various vitamins (Charles and Huston, 1972; Jones, 1986; Nir *et al.*, 1978; 1981), enzymes (Willingham *et al.*, 1961; Inborr and Bedford, 1994), lysine (Dale, 1992), and fungicides (Paster *et al.*, 1985). However, studies on stability of antibiotics to pelleting or in the presence of possible interfering factors have been limiting. McGinnis and Stern (1953) fed procaine penicillin, diamine penicillin, and chlortetracycline (CTC) in mash and pellet forms to turkeys and concluded on the basis of growth response that the antibiotics were not sufficiently destroyed by pelleting to affect growth rates. Watkins *et al.* (1999) demonstrated that BMD and CTC assays in a swine diet were not affected by pelleting, and remained within assay specifications for up to 13 weeks of storage after pelleting.

Both of the pellet binders used in this study are based on calcium lignosulfonate. Previous research has demonstrated that inclusion of calcium lignosulfonate as a pellet binder had no adverse effect on performance of broilers (Proudfoot and DeWitt, 1976; Proudfoot *et al.*, 1979; Proudfoot and Hulan, 1980; Waldroup *et al.*, 1981). Results of the present study indicate that the pellet binders Ameri-Bond 2x (calcium lignosulfonate) and Pel-Stik do not interfere with the assay of BMD in a broiler grower diet, and that pelleting of the feed does not result in destruction of BMD activity. The BMD assay results of pelleted feed, stored at ambient temperature (25° C) and ambient humidity (over 13 weeks), or elevated temperature (37° C) and ambient humidity (over 4 weeks), demonstrate that the pellet binders Ameri-Bond 2x (calcium lignosulfonate) and Pel-Stik do not affect the stability of BMD in feed. The use of BMD in feeds containing pellet binders will allow the medication to be delivered to the animal and allow the producer to realize all the advantages of high quality pelleted feed.

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References

Briggs, G.M. and M.R.S. Fox, 1956. Vitamin A deficiency in chicks produced by adding high levels of bentonite to synthetic diets. Poult. Sci., 35:570-576.

Charles, O.W. and T.M. Huston, 1972. The biological activity of vitamin K materials following storage and pelleting. Poult. Sci., 51:1421-1427.

Dale, N.M., 1992. Pelleting effects on lysine bioavailability in diets containing dried bakery product. J. Appl. Poult. Res., 1:84-87.

Inborr, J. and M.R. Bedford, 1994. Stability of feed enzymes to steam pelleting during feed processing. Anim. Feed Sci. Tech., 46:179-196.

Jones, F.T., 1986. Effect of pelleting on vitamin A assay levels of poultry feeds. Poult. Sci., 65:1421-1422.

Mathers, J.J. and A. D. Desai, 1995. Bioassay strain development for the analysis of bacitracin in bacitracin/chlortetracycline combinations. J. Industrial Microbiol., 15: 449-452.

McGinnis, J. and J.R. Stern, 1953. Effect of the feed pelleting process on growth response of poults to antibiotic supplements. Poult. Sci., 32:1036-1038.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Nir, I., R. Cohen and I. Kafri, 1981. Determination of vitamin A stability in mash and pelleted feeds by a biological procedure. Poult. Sci., 60:1022-1025.

Nir, I., I. Kafri and R. Cohen, 1978. Effect of pelleting on the vitamin K activity of various vitamin K3 preparations in chicks. Poult. Sci., 57:206-209.

Paster, N., I. Bartov and A. Perelman, 1985. Studies of the fungistatic activity of antifungal compounds in mash and pelleted feeds. Poult. Sci., 60: 1673-1677.

Proudfoot, F.G. and W.F. DeWitt, 1976. The effect of the pellet binder "Lignosol FG" on the chicken's digestive system and general performance. Poult. Sci., 55:629-631.

Proudfoot, F.G. and H.W. Hulan,1980. The effect of Nutri-Bond as a pellet binder in chicken broiler diets. Poult. Sci., 59:659-661.

Proudfoot, F.G., H. W. Hulan and W.F. DeWitt, 1979. The effects of using 0, 1.0, 1.5, and 2.0% "Lignosol FG" as a pellet binder in broiler chicken finisher diets. Poult. Sci., 58:87-89.

SAS Institute, Inc., 1988. SAS/STAT® User's Guide, Release 6.03 Edition. SAS Institute, Inc., Cary, NC.

Sibbald, I.R. and M.S. Wolynetz, 1989. Research note: Pellet binder and steam pelleting as nitrogen-corrected true metabolizable energy contributors: and example of the statistics used to evaluate a component of a mixture. Poult. Sci., 68:1299-1302.

Waldroup, P.W., S.J. Ritchie and B.E. Ramsey, 1981. The effects of lignin sulfonate pellet binder on pellet quality and feeding value for broilers. Arkansas Mimeo Series 290, Arkansas Agricultural Experiment Station, Fayetteville, AR.

Watkins, S.E., P. W. Waldroup, J. P. Peters and A. D. Desai, 1999. Stability of Bacitracin methylene disalicylate and chlortetracycline in combination in a pelleted pig feed. Anim. Feed Sci. Tech, 81:35-42.

Willingham, H.E., R.E. Fry, K.C. Leong, L.S. Jensen and J. McGinnis, 1961. Studies on stability of enzyme supplements to pelleting, long storage, and other treatments. Poult. Sci., 40:854-857.