Dietary Vitamin K_1 Requirement and Comparison of Biopotency of Different Vitamin K Sources for Young Turkeys[1]

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ABSTRACT In a preliminary experiment, the inclusion of vitamin K_1 (K_1) at a dietary level of 0.1 mg/kg was as effective as 1 or 2 mg/kg in reducing plasma prothrombin time (PT). To obtain an estimate of the dietary K_1 requirement and to compare the biopotency of different vitamin K sources for poults, three additional experiments were conducted. In Experiment 1, an incomplete factorial arrangement of treatments was used in which five dietary concentrations of K_1 (0, 0.1, 0.25, 0.5, or 2.0 mg/kg) were tested and two concentrations of neomycin (0 or 75 mg/L) in drinking water were used in conjunction with 0, 0.1, and 0.5 mg of K_1/kg of diet. Thus, we used a total of eight treatments. Each treatment was given to two pens of poults, with eight poults per pen. Prothrombin time and prothrombin concentration (PC) in plasma were not influenced by inclusion of neomycin in drinking water. The K_1 requirement was estimated, on the basis of PT and PC, to be 0.099 and 0.13 mg/kg, respectively, in Experiment 1. Dietary K_1 concentrations tested in Experiment 2 were 0, 0.08, 0.31, or 0.44 mg/kg. A similar protocol to that of Experiment 1 was used in this experiment. The results of Experiment 2 indicated that the dietary K_1 requirement was 0.079 mg, based on the influence of dietary K_1 on PT. In Experiment 3, dietary treatments consisted of the equivalent of 0.22, 0.55, or 1.11 μM of menadione equivalent/kg from vitamin K_1, menadione dimethypyrimidinol bisulfite (MPB) or menadione nicotinamide bisulfite (MNB), respectively, and a control without supplementation of any vitamin K source. The results of Experiment 3 showed that the biopotency of K_1 was greater than that of MPB or MNB. The biopotencies of MPB and MNB were similar, although MNB was more potent in reducing plasma PT when supplemented at the level of 0.1 mg of menadione/kg. A nadir of PT and a plateau of PC were evident with a dietary supplementation of MPB or MNB at a level of 0.25 mg of menadione/kg. Results of this research show that the dietary K_1 requirement of young turkeys is in the range of 0.079 to 0.13 mg/kg, and ingestion of neomycin did not affect estimates of the requirement. The biopotency of vitamin K_1 in reducing plasma PT and increasing plasma PC was greater than that of MPB or MNB. The biopotency of MNB was greater than that of MPB when menadione supplementation was equivalent to 0.10 mg of K_1/kg.

(*Key words*: vitamin K_1, plasma prothrombin time, plasma prothrombin, vitamin K_1 source, turkey)

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INTRODUCTION

Vitamin K is a general term used for a series of compounds with a structure of polyisoprenoid-substituted naphthoquinone. It includes vitamins K_1 (phylloquinone) (K_1) and K_2 (menaquiones), which originate from plants or bacteria, respectively, and a group of synthetic menadione derivatives such as menadione sodium bisulfite, etc. The K vitamins are essential cofactors for microsomal vitamin K-dependent carboxylases, which catalyze a post-translational conversion of glutamyl residues in the nascent precursors to γ-carboxyglutamyl residues (GLA) in their respective mature proteins (Suttie, 1985).

The vitamin K requirement of animals such as turkeys is met by both dietary sources and from microbial synthesis in the gastrointestinal tract. Intake of broad-spectrum antibiotics, such as neomycin, has been shown to reduce the production of menaquinones by microbial flora in the intestinal tract and has resulted in symptoms of vitamin K deficiency in humans when the dietary vitamin K source was very limited (Udall, 1965; Frick et al., 1967).

There is very little information in the literature on the vitamin K requirement of turkeys. On the basis of prothrombin times (PT) of young turkeys, Griminger (1957) reported that when menadione sodium bisulfite or mena-

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Abbreviation Key: K_1 = vitamin K_1 (phylloquinone); MBSC = menadione sodium bisulfite complex; MK-4 = menaquionone-4; MPB = menadione dimethylpyrimidinol bisulfite; MNB = menadione nicotinamide bisulfite; PC = prothrombin concentration; PT = prothrombin time.

dione was used as a source of vitamin K, the requirements were 1.1 and 1.76 mg/kg diet, respectively. The dietary vitamin K requirement of young turkeys listed by the National Research Council (1994) is actually a menadione sodium bisulfite requirement based on the data of Griminger (1957), and no requirement for vitamin K_1 is given. Griminger and Donis (1960) reported that the K_1 requirement of chicks fed a purified diet was 1 mg/kg of diet. Nelson and Norris (1958, 1960) estimated the K_1 requirement for chicks varied from 0.4 to 0.6 mg/kg diet. Results of previous research in our laboratory (Jin et al., 2001) that involved use of different dietary concentrations of K_1 indicated that young turkeys might require approximately 0.1 mg of K_1/kg. The objectives of the research reported here were to determine the K_1 requirement of young turkeys by using plasma PT and plasma prothrombin concentration (PC) as primary criteria and to evaluate the biopotency of menadione dimethypyrimidinol bisulfite (MPB) and menadione nicotinamide bisulfite (MNB).

MATERIALS AND METHODS

Diets and Treatments

In Experiment 1, we used an incomplete factorial arrangement of treatments in which five dietary concentrations of K_1 (0, 0.1, 0.25, 0.5, or 2.0 mg/kg) were tested and two concentrations of neomycin (0 or 75 mg/L) in drinking water were used in conjunction with 0, 0.1, and 0.5 mg of K_1/kg of diet. Thus, a total of eight treatments was involved. Each treatment was given to two pens of poults, with eight poults per pen. Composition of the low K_1 basal diet is shown in Table 1. Each treatment was fed to two pens with eight poults per pen from Days 1 to 7. The poults were housed in brooder batteries equipped with wire-mesh floors. Diets were fed in mash form. Dietary K_1 concentrations tested in Experiment 2 were 0, 0.10, 0.25, 0.50, and 2.0 mg/kg, on a calculated basis. Results of analysis of the diets by a commercial laboratory[3] showed concentrations of 0.08, 0.31, 0.44, and 1.8 mg of K_1/kg, respectively. Thus, determined K_1 values were in reasonable agreement with calculated values. Each treatment was fed to two pens with 10 poults per pen from Days 1 to 7. Ten dietary treatments were used in Experiment 3. One treatment, the control diet, contained no source of supplemental vitamin K. Nine treatments were obtained from a complete factorial arrangement of three concentrations of a vitamin K source and three sources of vitamin K. One series of the factorial consisted of diets supplemented with 0.1, 0.25, or 0.50 mg of K_1/kg. The remaining six treatments were obtained by supplementing diets with MPB or MNB at concentrations equivalent, on a molar basis, to the concentration of menadione contributed by K_1 in the three diets described above. The sources of MPB and MNB contained 34% menadione. Thus, concentrations of these two sources of vitamin K were 0.112, 0.281, and 0.562 mg/kg of diet. These concentrations were equivalent, on a molar basis, to 0.1, 0.25, and 0.50 mg of K_1/kg of diet. All the dietary treatments were based on the diet shown in Table 1. Each treatment was fed to one pen of nine poults from 1 to 14 d.

TABLE 1. Composition of the basal diet

Ingredients	Content
	(g/kg)
Soybean meal (48% CP)	567.03
Corn starch	267.70
Sunflower meal	80.00
Stripped corn oil	39.02
Dicalcium phosphate	23.83
Limestone	12.67
Vitamin premix[1]	3.00
Mineral premix[2]	3.00
DL-Methionine	2.00
NaCl	1.50
BMD[3]	0.25
Calculated nutrient composition	
ME, kcal/kg	2,850
CP, %	28.5
TSAA, %	1.05
Lysine, %	1.69
Calcium, %	1.20
Available phosphorus, %	0.60
Vitamin K_1, mg/kg	<0.02[4]

[1]Provided per kilogram of diet: vitamin A (retinyl acetate), 8,190 IU; dl-α-tocopheryl acetate, 30 IU; vitamin D_3 (cholecalciferol), 1,650 IU; vitamin B_{12}, 15.9 μg; pantothenic acid, 13.2 mg; niacin, 75 mg; choline, 509 mg; folic acid, 2.4 mg; biotin, 0.27 mg; pyrodixine HCl, 6.0 mg; thiamine mononitrate, 2.4.

[2]Supplied per kilogram of diet: Mn, 70 mg; Zn, 40 mg; Fe, 37 mg; Cu, 6 mg; Se, 0.15 mg; NaCl (I), 2.60 g.

[3]Bacitracin dimethylene salicylate, A. L. Pharma, Inc., Ft. Lee, NJ 07024.

[4]Detection limit for vitamin K_1, CN Laboratory, Courtland, MN 56021.

Animals

One-day-old Nicholas male poults were obtained from a commercial hatchery and were randomly assigned to each treatment pen. The poults were kept in heated, thermostatically controlled batteries with raised wire floors. Water and feed were provided ad libitum. Water troughs were cleaned daily to reduce the possibility of production of menaquiones by microbial fermentation. Excreta in feeders, if any, were removed daily. Droppings under the battery pens were removed frequently to minimize coprophagy. In Experiments 1 and 2, body weight and feed consumption data were recorded when poults were 7 d of age, and blood samples were obtained from each poult in every pen. Citrate was used as anticoagulant for the blood samples from which plasmas were used to determine PT and PC. In Experiment 1, a second blood sample was taken from every poult, and heparin was used as an anticoagulant to facilitate the determination of plasma K_1 and menaquinone-4 (MK-4) concentrations. In Experiment 3, body weight and feed consumption were monitored weekly. A blood sample was obtained from every poult of each pen at 14 d of age. A similar protocol,

[3]CN Laboratory, Courtland, MN 56021.

as described in Experiments 1 and 2, was used to obtain plasmas to determine PT and plasma PC.

Analytical Procedures

Plasma K_1 and MK-4 concentrations were determined by colleagues at the University of Wisconsin[4] using the HPLC method of Haroon et al. (1986). The following is a brief description of the method. Plasma samples were extracted through a liquid phase with a ratio of plasma, water, ethanol, and hexane at 1:2:3:9. After centrifugation, the organic layer at the top of the supernatant was collected and evaporated to dryness. The dried sample was resuspended in hexane, filtered through a silica Sep-Pak cartridge, and eluted with a 3% mixture of ether in hexane. K_1 and MK-4 were analyzed on an HPLC with a Zorbax C_{18} reverse-phase chromatography column, a zinc reduction column, and a fluorescent detector. The zinc reduction column reduced vitamin K compounds to their fluorescent hydroquinones. The mobile solvent used was 15% methylene chloride in methanol with 5 mL/L reductive solvent additive. The rate of solvent flow was set to 1.0 mL/min, and pressure was 7,757 cm mercury. Excitation was performed at 244 nm, and emission was monitored at 418 nm. The internal standard used was di-hydrophylloquinone.

One-stage PT was determined manually using a commercial thromboplastin preparation, Simplastin® Excel.[5] Thromboplastin contained in this preparation was obtained from rabbit brain. The plasma prothrombin concentration was determined by an amidolysis assay (Shah et al., 1984). The principle of the method was, briefly, plasma prothrombin was activated to thrombin with a commercial preparation of thromboplastin, Simplastin® Excel.[5] The thrombin that was produced catalyzed an amidolysis reaction of a specific chromogenic peptide substrate, Pefachrome TH,[6] to release p-nitroaniline. The concentration of p-nitroaniline was determined spectrophotometrically. A standard curve was developed with thrombin (EC 3.4.21.5).[7]

Statistical Analysis

Although PT and PC data were obtained on all poults within each pen in Experiments 1, 2, and 3, pens were used as the experimental unit for statistical analysis according to a heirarchal classification. Data obtained from factorial part of Experiment 1 were analyzed by a two-way ANOVA to determine the main effects and interaction between neomycin inclusion in drinking water and dietary K_1 concentration using the general linear model (SAS Institute, 1996). Because neomycin treatment of the drinking water had no effect on criteria of dietary K_1 adequacy in Experiment 1, data obtained for the five concentrations of dietary K_1 were subjected to regression analysis (one-slope or two slope methods of Robbins, 1986) to obtain an estimate of the dietary K_1 requirement. One-slope and two-slope regression analyses also were used to evaluate data of Experiment 2. Data of Experiment 3 were analyzed by two-way ANOVA, excluding data from the control group, to determine the main effects or interactions of dietary concentrations of vitamin K sources and concentrations of the sources, using individual poults as the experimental units. These data also were analyzed by a one-way ANOVA. Where appropriate, differences among main effect means (Experiment 1) and treatment means (Experiments 1, 2, and 3) were determined by using least significant difference tests.

TABLE 2. Effect of neomycin in drinking water and dietary supplemental vitamin K_1 (K_1) on plasma prothrombin time (PT) and plasma prothrombin concentration (PC); Experiment 1

Item	Supplemental K_1 (mg/kg)	PT (s)	PC (NIH unit)
Neomycin (mg/L)	0	124.6[2]	44.3
0	0.1	112.5	104.0
	0.5	93.6	102.6
	0	129.7	31.4
75	0.1	106.8	103.8
	0.5	101.2	120.8
SEM		7.7	17.2
Means of main effects			
Neomycin			
0		109.7	83.6
75		112.1	85.3
K_1			
0		126.9[a]	37.8[a]
0.1		109.2[b]	103.9[b]
0.5		96.9[b]	111.7[b]
		——— (*P*) ———	
Sources of variation			
Neomycin		0.41	0.93
K_1		0.04	0.05
Neomycin × K_1		0.42	0.95

[a,b]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]PC is expressed in terms of thrombin activity in NIH units. NIH unit is obtained by direct comparison to an NIH Thrombin Reference Standard, Lot J, Sigma, St. Louis, MO 63178.

[2]Means of two pens per treatment, eight poults per pen.

RESULTS

Experiment 1

Analysis of data obtained from the factorial part of the experiment showed that inclusion of neomycin in the drinking water increased ($P \leq 0.01$) body weight gain but had no effect on feed efficiency (data not shown). Neither PT nor PC concentration in plasma was influenced by neomycin in drinking water, whereas inclusion of 0.1 mg of K_1/kg of diet decreased plasma PT and increased plasma PC (Table 2). Analysis of data obtained from supplementation of diets with increments of K_1, excluding

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[5]Organon Teknika Corp., Box 15969, Durham, NC 27704-0969.

[6]Centerchem, Inc., 225 High Ridge Road, Stamford, CT 06905.

[7]Sigma Diagnostics, Inc., St. Louis, MO 63178.

TABLE 3. Effect of dietary supplemental vitamin K_1 (K_1) on plasma prothrombin time (PT), prothrombin concentration (PC), and plasma K_1, menaquione-4 (MK-4) concentrations in young turkey poults; Experiment 1

Supplemental K_1 (mg/kg)	PT (s)	PC[1] (NIH unit)[2]	Plasma K_1	MK-4
			(ng/mL)	
0	124.6[a,3]	44.3[a]	0.27	0.83
0.1	112.5[ab]	104.0[b]	0.66	1.27
0.25	116.1[b]	92.8[b]	1.36	1.94
0.5	94.6[c]	102.6[b]	1.95	1.94
2.0	90.6[c]	101.7[b]	7.23	6.21
SEM	5.61	16.3	0.883	0.432
		(*P*)		
Source of variation				
K_1	0.01	0.03	0.04	0.03

[a–c]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]PC is expressed in terms of thrombin activity in NIH units.

[2]NIH unit is obtained by direct comparison to an NIH Thrombin Reference Standard, Lot J, Sigma, St. Louis, MO 63178.

[3]Means of two pens per treatment, eight poults per pen.

treatments in which neomycin was added to the drinking water, showed that plasma PT decreased as dietary K_1 increased (Table 2). Plasma PC concentration was increased ($P = 0.03$) when 0.1 mg K_1 was included per kilogram of diet, and use of additional K_1 supplementation caused no further increase in plasma PC. The dietary K_1 requirements estimated using the broken-line method were 0.099 mg and 0.13 mg/kg, based on plasma PT and PC, respectively. Plasma K_1 and MK-4 concentrations increased linearly as the dietary K_1 level increased (Table 3).

Experiment 2

Dietary K_1 concentration had no effect on body weight gain, feed intake, or efficiency of feed utilization (data not shown). However, plasma PT was decreased by dietary K_1 supplementation (Table 4). Supplementation of 0.08 mg of K_1/kg in the basal diet was as effective as supplementation with 0.31, 0.44, or 1.8 mg of K_1/kg in reduction of PT. The estimated dietary K_1 requirement of poults using the broken-line method was 0.079 mg/kg, based on PT.

TABLE 4. Effect of dietary K_1 on plasma prothrombin time (PT); Experiment 2

Supplemental K_1 (mg/kg)	PT (s)
0	130.4[1,a]
0.08[2]	88.9[b]
0.31	98.7[b]
0.44	100.7[b]
1.80	92.3[b]
SEM	7.59
	(*P*)
Source of variation	
K_1	0.02

[a,b]Means within a column with no common superscript differ significantly, ($P < 0.05$).

[1]Means of two pens per treatment, 10 poults per pen.

[2]As determined by laboratory analysis, CN Laboratory, Courtland, MN 56021.

Experiment 3

Supplementation of the control diet with 0.10 mg of K_1 decreased plasma PT and increased plasma PC (Table 5). Supplementation with 0.25 or 0.50 mg of K_1 did not cause additional changes in these criteria of vitamin K status.

TABLE 5. Effect of supplementation of diets with vitamin K_1 (K_1), menadione dimethylpyrimidinol (MPB), or menadione nicotinanimide bisulfite (MNB) or plasma prothrombin time (PT) and plasma prothrombin concentration (PC) of 14-d-old turkeys; Experiment 3

Item	Dietary concentration vitamin K source (mg/kg)	PT (s)	PC (NIH units)[1]
Source of vitamin K			
Control	None	127[a,2]	124[d]
K_1	0.10	77.7[cd]	254[ab]
MPB	0.112	132.7[a]	154[cd]
MNB	0.112	99.4[b]	180[cd]
K_1	0.25	69.6[d]	250[ab]
MPB	0.28	89.3[bc]	204[bc]
MNB	0.28	82.7[cd]	210[bc]
K_1	0.50	82.1[cd]	296[a]
MPB	0.56	78.9[cd]	288[a]
MNB	0.56	75.9[cd]	240[ab]
SEM	. . .	4.1	7.9
		(*P*)	
Source of variation[3]			
Vitamin K source (S)		0.001	0.003
Concentration of source (C)		0.001	0.001
S × C		0.001	0.22

[a,b]Means with no common superscript letter differ significantly ($P \leq 0.05$). These comparisons were based on analysis of data of all 10 treatments.

[1]NIH units were obtained by comparison with an NIH Thrombin Reference Standard, Lot J, Sigma, St. Louis, MO 63178.

[2]Means of nine poults per dietary treatment.

[3]Results of two-way ANOVA, excluding data of the control group.

When MNB was included in the diet at a menadione concentration equivalent to 0.10 mg of K_1/kg, plasma PT also was decreased, whereas supplementation with the equivalent concentration of menadione from MPB did not decrease plasma PT. Neither MNB nor MPB changed plasma PC, as compared with the controls, when used at menadione concentrations equivalent to 0.10 mg of K_1/kg. Plasma PT and PC tended to plateau when 0.25 or 0.50 mg of K_1/kg, or menadione equivalent concentrations of MPB or MNB, were included in the diets.

DISCUSSION

Data on plasma PC and plasma PT reported here generally show that the dietary K_1 requirement of 7- to 14-d-old turkeys is approximately 0.10 mg/kg of diet. One exception among these data occurred in Experiment 1, in which 0.25 to 0.50 mg of K_1/kg were needed to minimize PT. However, plasma PC was maximized in Experiment 1 when poults were fed 0.10 mg of K_1/kg of diet, and Kindberg and Suttie (1989) reported that plasma PC is a more specific and sensitive indicator of vitamin K status of rats than is PT. Information on the K_1 requirement of poults is lacking in the literature. Griminger (1957) reported that supplementation of a diet deficient in vitamin K with 1.76 mg of menadione, or an equal amount of menadione sodium bisulfite complex, was needed to minimize plasma PT of 2-wk-old turkeys. These data served as the basis for the vitamin K requirement listed by the National Research Council (1994).

The dietary K_1 requirement of 7- to 14-d-old poults reported from the current research (0.10 mg/kg) is substanitally less than estimates of the K_1 needs of chicks. Nelson and Norris (1960) found that the K_1 requirement of 2- to 4-wk-old chicks fed a low K_1, purified diet was 0.53 mg/kg, whereas Griminger and Donis (1960) reported that 3-wk-old chicks also fed a purified diet required about 1 mg of K_1/kg of diet. These researchers used minimum plasma PT as the criterion of K_1 status. Data reported by Scott (1966) tended to confirm the K_1 requirement observed by Nelson and Norris (1960), in that supplementation of a purified, low vitamin K diet with 0.18 to 0.36 mg of K_1 was needed to minimize plasma PT. Reasons are not evident for the greater dietary K_1 requirement determined for chicks than the K_1 requirement of poults reported herein. Chick requirements were determined by using purified diets, whereas cornstarch was the only purified ingredient in diets of the current studies. Laboratory analysis, however, showed that our basal diet contained less than 0.02 mg of K_1/kg.

In the current research, thromboplastin obtained from rabbit brain was used in the prothrombin determination. Griminger (1957), Griminger and Donis (1960), Nelson and Norris (1960), and Scott (1966) used thromboplastin extracts from chicken brain. Generally, prothrombin times in the current study were longer than those reported by researchers working with chicks, and the differences could have been related to some degree of species specificity of thromboplastin. Nevertheless, relatively clear-cut breakpoints in plasma PT, indicative of adequate K_1 status of poults, were observed in the current studies. Furthermore, the breakpoints at which plasma PC plateaued, also indicative of adequate K_1 status, were evident and consistently showed that poults required only about 0.10 mg of K_1/kg of diet. Suttie et al. (1988) reported that the K_1 requirement of humans was approximately 1 μg/kg body weight per day. If it is assumed that the K_1 requirement of poults per unit of metabolic body size would be similar to that of humans, the calculated K_1 requirement of 7- to 14-d-old poults should be less than 0.01 mg of K_1/kg of diet. Thus, the dietary requirement of 0.10 mg of K_1/kg, as determined in the current studies, seems reasonable.

The results of Experiment 1 showed that giving poults 75 mg of neomycin/L of drinking water did not affect an estimate of dietary K_1 needs. This finding suggests that biosynthesis of vitamin K in the intestinal tract had little influence on K_1 status of poults. Griminger (1957) indicated that synthesis of vitamin K in the intestinal tract was relatively unimportant in supplying this vitamin to chicks, whereas coprophagy could provide a source of vitamin K. Management of poults used in the studies reported here minimized opportunities for coprophagy.

Vitamin K_1 supplementation of the low K_1 diet had no effect on weight gain or feed consumption of poults in the current research. Feeding the K_1 deficient diet to poults for 14 d also did not result in any mortality, and no visible signs of subcutaneous or internal hemmorhages were observed. These observations agree with those described by Griminger (1957), who stated that in earlier research, "Chicks on similar rations usually exhibit subcutaneous hemmorhages within three weeks. It is not uncommon to obtain chicks severely weakened by massive internal hemmorhages, and mortality, often times due to brain hemmorhages, occurs. It was, therefore, remarkable that the poults on this series of experiments rarely showed any external signs of hemmorhages, and that few death losses were encountered. The only symptom observed, besides prolonged prothrombin times, was lesions in the gizzard." Griminger (1957) also stated that the gizzard lesions did not seem to be related to vitamin K nutriture.

Because of the cost of K_1 and its instability in premixes and diets, less expensive, more stable sources of vitamin K have been used in poultry feeds. These sources include menadione bisulfite complex (MBSC), MPB, and recently, MNB. Research done to compare the biopotency of MBSC and MPB as vitamin K sources has shown that MPB is 1.9 to 2 times as potent as MBSC (Griminger, 1965; Dua and Day, 1966). However, information is lacking on the relative vitamin K biopotency of MPB and MNB for young turkeys. Results of Experiment 3 showed that these two dietary vitamin K sources were of similar potency when supplemented at levels equivalent to 0.25 or 0.50 mg of K_1/kg of diet. When MPB and MNB were added at levels equivalent to 0.10 mg of K_1/kg, the biopotency of MNB was greater than that of MPB, but only in terms of decreased PT.

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