# Arsanilic acid, sodium salicylate and bromide salts as potential growth stimulants for pigs receiving diets with and without copper sulphate

By R. S. BARBER, R. BRAUDE AND K. G. MITCHELL
National Institute for Research in Dairying, Shinfield, Reading RG2 9AT

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- 1. Results are presented of three experiments involving 112 individually fed pigs, covering the growing period between 20 and 90 kg live weight, in which the potential performance-stimulating properties of three different compounds were studied in relation to those of a dietary supplement of 250 mg Cu/kg diet.
- 2. The three compounds studied and the levels of supplementation used (mg/kg diet) were: Expt 1 arsanilic acid (100), Expt 2 sodium salicylate (200), Expt 3 a mixture of bromide salts (200); each was added to the diets either alone or with 250 mg Cu/kg diet given as CuSO<sub>4</sub>.5H<sub>2</sub>O.
- 3. The diets were given wet, 'to appetite' twice daily up to a daily maximum of 3 kg feed/pig. Measurements of length and of back-fat thickness at a number of points were made on all carcasses at slaughter.
- 4. Arsanilic acid and sodium salicylate alone gave growth responses similar to those obtained with Cu alone, whereas the bromide salt had no effect on performance. None of the three compounds when added to diets in conjunction with 250 mg Cu/kg diet gave results superior to those obtained with the Cu supplement alone.
- 5. When added to the diets alone, none of the three compounds tested nor Cu had any marked effect on any of the carcass measurements.
- 6. Arsanilic acid reduced total liver weight and markedly reduced the large increase in total liver Cu stores that resulted from supplementation of the diets with 250 mg Cu/kg diet.

Barber, Braude & Mitchell (1965) reported the results of three experiments with growing pigs in which the potential growth-promoting activities of (a) zinc bacitracin (a narrow-spectrum antibiotic), (b) Protamone (an iodinated casein containing 1% thyroxine activity) and (c) Emtryl (dimetridazole) were investigated in relation to the well-established growth-promoting effect of a dietary supplement of 250 mg Cu/kg diet. None of the three materials had any effect on the performance of the pigs that suggested it would be either preferable to copper sulphate as a growth-promoter, or of value when added to a diet in addition to the Cu supplement. In the present paper, the results of investigations on three further materials are reported.

### EXPERIMENTAL

All the experiments were similar in design and, in each, pigs were given three treatments and compared with a control group of pigs. The treatments were the addition to a control diet of either copper sulphate (providing 250 mg Cu/kg diet) alone, or one of the three materials being investigated alone, or copper sulphate and the material under investigation together.

## Materials investigated

Arsanilic acid. This is one of several organic arsenicals derived from phenylarsonic acid in which the arsenic present is in the pentavalent form. In the years following the work of Morehouse (1949) with poultry and of Carpenter (1951) with pigs, which suggested that organic arsenicals had a potential growth-promoting property, a considerable volume of experimental evidence was reported in the literature, mainly from the USA, confirming that continuous supplementation with arsenicals at a low level (up to 100 mg/kg diet) frequently improved the performance of growing pigs (see Anonymous (1956) for a general review of some of the earlier work with various species and Lennig Chemicals Ltd (1970) for a more recent review of a useful selection of the reported work with arsanilic acid in pigs, poultry and turkeys). The therapeutic value of some arsenicals when administered to pigs under certain conditions at high levels (up to 250 mg/kg diet) for short periods of time is also now well established, and their safety both for the animal itself and the potential consumer of the meat from such animals is well documented (see Frost, 1963, 1967).

Sodium salicylate. The antiseptic properties of salicylic acid (o-hydroxybenzoic acid) are well known, while its acetic acid ester (acetylsalicylic acid) is more widely known as 'aspirin'. Following the report by Rechka & Kalous (1964) that a supplement of either sodium salicylate (100 mg/kg diet) or of sodium acetylsalicylate (500 mg/kg diet) appeared to increase the growth rate of pigs, further papers from Czechoslovakia were subsequently published which, in the main, substantiated these findings (Müller & Čulík, 1965; Herzig & Najman, 1966; Šatava, Štrunc, Rozman, Čulík & Müller, 1966; Müller, Šatava, Vrchota & Štrunc, 1967; Rozman, 1968 a, b: Rozman & Hejzlar, 1970). Most of the reports were concerned with sodium salicylate, and levels giving responses ranged from 100 to 700 mg/kg diet.

Bromide salts. Eidrigevich, Osipov, Zaplatnikov, Bazarnyi, D'yakova & Rumyantsev (1967) published results of several trials with growing pigs and fattening cattle given daily doses of a mixture of bromide salts. They reported that a daily intake of 5 mg/kg live weight of a mixture of sodium, potassium and ammonium bromides increased the rate of growth of the pigs. They noted an inhibition of sexual function while the bromides were being administered but, after withdrawal, representatives of both sexes were successfully used for breeding.

## Pigs and procedure

For all three experiments, enzootic-pneumonia-free Large White pigs in litter-mate groups, balanced as far as possible for sex, were used. The pigs were selected after weaning (at 8 weeks of age) and were on experiment from 9–10 weeks of age (average initial weight 20 kg) to 90 kg live weight. They were housed in a Danish-type piggery in pens of four; in any one pen, all four pigs received the same treatment, so there was no direct contact between pigs on different treatments. There were twelve pigs/treatment in Expt 1 (arsanilic acid) and eight pigs/treatment in both Expt 2 (sodium salicylate) and Expt 3 (bromide salts).

All pigs were individually fed twice daily, 3.0 l (Expt 1) or 2.5 l (Expts 2 and 3)

water/kg diet being added immediately before feeding, and at each meal the pigs were given access to the troughs for 30 min. To commence with, the daily allowance was 1.05 kg feed for a pig weighing 20 kg; thereafter it was increased by 0.1 kg for each pig whenever its trough was completely cleared on 2 successive days, up to a maximum of 2.95 kg in Expts 1 and 3 and up to 3.0 kg in Expt 2. If a pig refused 20% or more feed on 2 successive days, the daily allowance was reduced by the estimated amount of the refused feed.

Table 1. Expts 1 and 2. Percentage composition of basal diets received by all pigs

	Up to 54 kg live weight	From 54 kg live weight to slaughter	
Barley meal	54	70	
Fine wheat offal	38	25	
White fish meal	7	3	
Limestone flour	I	0.75	
Steamed bone flour	_	r	
Salt		0.25	
Rovimix* (g/100 kg diet)	10	5.6	

\* Roche Products Ltd, containing 50 000 i.u. vitamin A and 12 500 i.u. cholecalciferol/g.

Table 2. Expt 3. Percentage composition of basal diets received by all pigs

	Up to 54 kg live weight	From 54 kg live weight to slaughter	
Barley meal	66.75	70.25	
Fine wheat offal	25	25	
White fish meal	7	3.2	
Mineral-vitamin supplement*	1.25	1.25	

\* Cooper Nutrition Products Ltd, supplying in the 12.5 kg added per 1000 kg diet:  $4 \times 10^6$  i.u. vitamin A,  $1 \times 10^6$  i.u. cholecalciferol, 2 g riboflavin, 8 g nicotinic acid, 6 g pantothenic acid, 2 mg cyanocobalamin, 100 g Fe, 70 g Zn, 25 g Mn, 5 g Cu, 1 g I, 0.5 g Co, 2.5 kg NaCl, 9.6 kg limestone flour.

The compositions of the basal diets given in Expts 1 and 2 are shown in Table 1, and in Table 2 the basal diets used in Expt 3 are given. In the interval between the completion of Expts 1 and 2 and the start of Expt 3, a number of changes were made in the composition of the standard control diets used in most of the feeding experiments with growing pigs at this Institute.

In all experiments, on treatments 2 and 4 (see Tables 3, 4 and 5) the copper sulphate supplement (CuSO<sub>4</sub>.5H<sub>2</sub>O) used was in the form of a finely ground powder and was added to the basal diets at the level of 1 g/kg diet (supplying 250 mg Cu/kg diet). The arsanilic acid used was provided as Pro-Gen Premix (Abbott Laboratories Ltd, Queenborough, Kent) containing 250 g arsanilic acid/2·27 kg (5 lb) in a limestone flour base, and was added to the basal diets in Expt 1 at a level to supply 100 mg arsanilic acid/kg diet for treatments 3 and 4 (see Table 3). In Expt 2, sodium salicylate was added to the basal diets at a level of 200 mg/kg diet for treatments 3 and 4 (see Table 4). For Expt 3, a bromide salt mixture consisting of equal parts of ammonium,

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potassium and sodium bromides as a finely ground powder was added to the basal diets for treatments 3 and 4 at a level of 200 mg/kg diet (see Table 5).

All experimental supplements except the bromide salt mixture were included in the diets throughout the experimental period. In Expt 3 the bromide salt supplement was withdrawn from the diet from the time the pigs reached a minimum live weight of 78 kg at the weekly weighing.

The pigs were weighed once a week and were sent to slaughter on the day after they had reached a minimum live weight of 80.5 kg at the weekly weighing; they were killed approximately 19 h after their last feed. Carcass length, maximum thickness of shoulder fat and minimum thickness of loin fat were measured in accordance with the procedure used for commercial grading. Fat thickness over the eye muscle (longissimus dorsi), at the last rib in two positions (fat 'C' and fat 'K', approx. 4 cm and 9-10 cm respectively from the mid-line of the back), was also measured by means of an introscope on all carcasses.

At slaughter, the livers were collected from eight of the twelve litter-mate pigs on each of the four treatments in Expt 1 and from all eight pigs on each of treatments 1, 2 and 4 in Expt 2; no livers were collected from pigs in Expt 3. After the livers had been weighed, a sample of the tissue adjacent to the bile duct was taken and stored at  $-20^{\circ}$ for subsequent determination of the Cu content; in unpublished earlier studies we established that the Cu concentration of a sample of liver taken from this position approximated closely to the mean Cu concentration of the whole liver. After digestion of the sample by a wet-ashing technique (Andrus, 1955), followed by an appropriate dilution, the Cu content of the solution was estimated by atomic absorption spectroscopy using a single beam instrument (Model A3000; Southern Instruments, Camberley, Surrey).

## Statistical methods

Standard analyses of variance were done on each of the variables determined in each of the three experiments. For the liver Cu concentrations, logarithmic transformations were used as the within-treatment variances were heterogeneous.

### RESULTS

Details of the results for Expts 1, 2 and 3 are shown in Tables 3, 4 and 5 respectively. All but one of the pigs in the three experiments grew normally for the full experimental period, the one animal being withdrawn from treatment 4 in Expt 1 on account of an abnormally slow rate of growth; missing values were substituted for the results for this pig.

## Expt 1. Arsanilic acid (Table 3)

Copper sulphate and arsanilic acid alone similarly and significantly improved the rate of growth of the pigs, but the interaction was large and there was no further improvement when both the supplements were added to the diet (treatment 4). Whereas Cu significantly improved the feed: gain ratio (feed conversion ratio or FCR) and had no effect on daily feed intake, arsanilic acid had no effect on FCR but significantly increased daily feed consumption. There was a significant interaction between

the two supplements for this latter variable, arsanilic acid increasing daily feed intake only in the absence of Cu in the diet.

There were no significant differences between any of the treatments in their effect on dressing percentage, carcass length or thickness of shoulder fat. Neither copper

Table 3. Expt 1 (arsanilic acid). Mean daily weight gain, feed conversion ratio (FCR), carcass measurements, liver weight and liver copper content of pigs on treatments 1-4 given no supplement, or a supplement of copper sulphate, or arsanilic acid, or both

(Twelve pigs/treatment on experiment from approximately 19 to 92 kg live weight; liver values based on eight pigs/treatment)

	iivei vai	aco basca o	in eight big	50/ treatment	,		
				4		Average	effect of:
				(Copper			
		2	3	sulphate+		Copper	Arsanilic
	r	(Copper	(Arsanilic	arsanilic	SEM	sulphate	acid
	(None)	sulphate)	acid)	acid)	(a)	(b)	(c)
Daily gain (kg)	0.66	0.69	0.70	0.69	0.013	(d)	(d)
FCR (kg feed/kg gain)	3.36	3.10	3.29	3.16	0.036	o·15***	-0.05NS
Daily feed intake (kg)	2.30	2.30	2.29	2.17	0.028	(d)	(d)
Dressing percentage	73.7	74.5	74.2	74.5	0.28	0.55NS	0.25NS
Carcass length (mm)	793	802	800	795	4.2	1.5NS	0.20NS
Shoulder fat (mm)	48.8	50.1	50.3	48.7	0.78	-0.15NS	0.05NS
Loin fat (mm)	29.2	28.1	30.8	25.8	0.84	(d)	(d)
Fat 'C' (mm) (e)	24· I	23.8	25.9	21.5	0.89	(d)	(d)
Fat 'K' (mm) (e)	27.8	27.2	29.8	25.0	0.89	(d)	(d)
Liver weight (g)	1350	1360	1250	1270	42.9	19·8NS -	- 91.7*
Liver Cu (ppm dry matter): (f)				·		·	
Logarithmic value	1.58	3.05	1.71	2.77	0.097	(d)	(d)
Derived mean	38	1116	52	588			_
Total Cu stores/liver (mg): (f)							
Logarithmic value	1.53	2.67	1.31	2.37	0.098	(d)	(d)
Derived mean	17	463	21	235			_

- (a) Based on 32 df (one pig withdrawn from treatment 4 and missing values substituted) with the exception of the liver weight and liver Cu values, for which there were 20 df (one missing value on treatment 4).
- (b) Treatments  $[(2+4)-(1+3)] \div 2$ . Significance levels: NS, P > 0.05; \*0.05 > P > 0.01; \*\*0.01 > P > 0.001; \*\*\* P < 0.001.
  - (c) Treatments  $[(3+4)-(1+2)] \div 2$ . Significance levels as in (b).
- (d) The interaction between the two dietary supplements was large and average effects were not calculated.
  - (e) See p. 384.
- (f) Logarithmic transformations were used in the analysis of variance, and both the logarithmic and derived means are shown.

sulphate nor arsanilic acid alone had any significant effect on the thickness of either loin fat or fat at position 'C' or 'K', but when the two supplements were given together there was a marked reduction in each of these three variables, the differences between values for treatments I and 4 being significant for the loin and fat 'K' measurements.

Copper sulphate had no effect on liver weight, but significantly increased Cu concentration in the liver in both the absence and presence of arsanilic acid (P < 0.001). Arsanilic acid significantly reduced total liver weight, but had no significant effect on the concentration of Cu in the liver although, in the presence of dietary copper

sulphate, both the concentration and total store of Cu in the liver were reduced by arsanilic acid (treatments 4 and 2), although the former difference was not quite significant at the 5% level.

## Expt 2. Sodium salicylate (Table 4)

Copper sulphate and sodium salicylate alone similarly and significantly increased rate of growth and improved FCR, although with sodium salicylate (treatment 3) the difference for FCR did not quite attain the 5% level of significance. These responses to either supplement in the absence of the other were greater, although not significantly so, than those obtained when both supplements were added to the diet. There were no significant treatment differences or interaction for any of the carcass values shown in Table 4 with the exception that sodium salicylate significantly increased the thickness of shoulder fat.

Table 4. Expt 2 (sodium salicylate). Mean daily weight gain, feed conversion ratio (FCR), carcass measurements, liver weight and liver copper content of pigs on treatments 1-4 given no supplement, or a supplement of copper sulphate, or sodium salicylate, or both

(Eight pigs/treatment on experiment from approximately 20 to 92 kg live weight)

(=-8.00 F.92) or com		4 (Copper				Average effect of:		
	ı (None)	2 (Copper sulphate)	3 (Sodium salicylate)	sulphate+	SEM (a)	Copper sulphate (b)	Sodium salicylate (c)	
Daily gain (kg)	0.74	0.80	0.79	0.76	0.013	(d)	(d)	
FCR (kg feed/kg gain)	3.11	2.93	3.00	3.04	0.038	(d)	(d)	
Daily feed intake (kg)	2.29	2.35	2.37	2.32	0.034	0.002NS	6 0.025NS	
Dressing percentage	75.9	76·o	75.7	75.9	0.30	0·1NS	-0.2NS	
Carcass length (mm)	824	817	834	818	6.2	-11.2NS	5.6NS	
Shoulder fat (mm)	37.2	34.0	38·4	39.0	1.13	-1.3NS	3.1*	
Loin fat (mm)	23.0	22.6	23.9	26·o	1.43	0·9NS	2·1NS	
Fat 'C' (mm) (e)	22.5	21.6	21.5	23.2	0∙96	o·6NS	0·4NS	
Fat 'K' (mm) (e)	24.0	22.9	23.1	24·I	1.18	-0·1NS	0·2NS	
Liver weight (g)	1350	1320	(g)	1320	37.2	_		
Liver Cu (ppm dry matter): (f)								
Logarithmic value	1.81	2.91	(g)	2.91	0.049	_		
Derived mean	65	805	(g)	805	_	_		

<sup>(</sup>a) Based on 21 df with the exception of the liver weight and liver Cu values, for which there were 13 df (one missing value on treatment 2).

Copper sulphate, given alone or together with sodium salicylate, had no significant effect on liver weight. Copper sulphate significantly increased the liver Cu concentration, and the addition of sodium salicylate to the high-Cu diet (treatment 3) had no significant effect on this increase.

## Expt 3. Bromide salts (Table 5)

Copper sulphate significantly increased rate of growth and improved FCR, whereas the bromide salts had no effect on either of these two variables. The performance of the pigs given both supplements tended to be inferior to that of pigs given Cu alone.

There were no significant treatment differences for any of the carcass measurements shown in Table 5, with the exception of carcass length.

<sup>(</sup>g) Samples not taken; average effects not determinable. For remaining footnotes see Table 3.

Table 5. Expt 3 (bromide salts). Mean daily weight gain, feed conversion ratio (FCR) and carcass measurements of pigs on treatments 1-4 given no supplement, or a supplement of copper sulphate, or bromide salts, or both

(Eight pigs/treatment on experiment from approximately 19 to 92 kg live weight; fat 'C' and 'K' values based on seven pigs/treatment)

				. 4		Average effect of:	
	ı (None)	2 (Copper sulphate)	3 (Bromide salts)	(Copper sulphate+ bromide salts)	SEM (a)	Copper sulphate (b)	Bromide salts (c)
Daily gain (kg)	0.74	0.82	0.75	0.78	0.013	0.055**	-0.017NS
FCR (kg feed/kg gain)	3.16	2.90	3.15	3.02	0.046	o·17**	o∙o6NS
Daily feed intake (kg)	2.35	2.36	2.32	2.37	0.032	0.04NS	0.004NS
Dressing percentage	76.5	75.9	76.6	76.4	0.2	-0.37NS	0.29NS
Carcass length (mm)	802	821	814	801	4.34	(d)	(d)
Shoulder fat (mm)	39.9	39.6	38.6	39.8	0.90	0.44NS	-0.57NS
Loin fat (mm)	25.8	25.4	25.6	23.9	1.01	- 1.06NS	- 1.62NS
Fat 'C' (mm) (e)	24.4	23.7	25.4	24.7	1.01	-0.72NS	1.00NS
Fat 'K' (mm) (e)	29.0	29.4	30.9	29.3	1.26	-0.57NS	o·86NS

<sup>(</sup>a) Based on 21 df with the exception of the fat 'C' and 'K' values, for which there were 16 df (one missing value on treatment 2 and one on treatment 3).

For remaining footnotes see Table 3.

### DISCUSSION

In all three experiments, the responses to the addition of 250 mg Cu/kg diet, namely growth responses of 5.5, 9 and 10% and improvements in FCR of 5, 5.5 and 8.25% in Expts 1, 2 and 3 respectively, were within the range normally obtained with this supplement in growing pigs (Braude, 1965; Wallace, 1967).

The growth responses to arsanilic acid in Expt 1 and to sodium salicylate in Expt 2 confirm the reports in the literature, reviewed earlier, of similar responses to both compounds. In contrast, the negative results obtained with the bromide salts in Expt 3 were not in accord with the beneficial effects reported by Eidrigevich et al. (1967). Several other workers have reported responses in growth rate to arsenicals without significant effects on FCR, although some improvement in the latter appears to be more usually observed.

The magnitude of the growth response to arsanilic acid or to sodium salicylate was similar to that obtained with copper sulphate. A few comparisons of 3-nitro-4-hydroxyphenylarsonic acid, added to the diet at the relatively low level of 15–30 mg/kg, with copper sulphate (0·5–1·0 g/kg diet) have been reported, and in every instance the mean growth response to the Cu supplement was greater than that observed with the arsenical (Foster, Speer, Hays & Catron, 1958; Ruszczyc & Glapś, 1960, 1963; Hawbaker, Speer, Hays & Catron, 1961).

No comparisons between salicylates and copper sulphate appear to have been reported, but, in connexion with the magnitude of the observed growth response to the supplement of 200 mg sodium salicylate/kg diet and its similarity to that obtained with copper sulphate in the experiment described here, it is of interest that recently Rozman (1968b) and Rozman & Hejzlar (1970) reported that, during an experimental

period of 111 d, growing pigs given an average dietary supplement of 700 mg sodium salicylate/kg grew 12% faster with a 9% better FCR than pigs given the salicylate at the level of 200 mg/kg diet used by us. In view of the relatively low cost of sodium salicylate, further investigation of its activity as a growth-promoting agent appears to be justified.

In spite of the significant increases in rate of growth obtained with either copper sulphate, arsanilic acid or sodium salicylate when added to the diet individually, there was clearly no synergistic effect when either of the two latter materials was given together with Cu. Our results with copper sulphate plus arsanilic acid are in agreement with those reported by Foster et al. (1958) and Hawbaker et al. (1961), although the responses obtained by both these sets of workers with the arsenical alone were very low in comparison with the 11–14% responses they obtained with copper sulphate alone.

The absence of any synergistic effect between Cu and either arsanilic acid or sodium salicylate parallels the results often observed in similar comparisons between Cu and certain antibiotics. At the same time, with Cu and antibiotics, synergistic effects have been observed under some conditions (Braude, 1965), and hence the present results with arsanilic acid and sodium salicylate do not preclude the possibility of the occurrence of synergistic effects with Cu under other unspecified conditions.

The responses in performance in these experiments were all obtained without detriment to the quality of the carcasses as indicated by the measurements of length and fat thickness, with the single exception of a suggestion that sodium salicylate slightly increased shoulder-fat thickness. The absence of any significant effect of Cu on carcass quality is in agreement with the majority of reports in the literature (Braude, 1965). The observation in Expt 1, that the addition of both Cu and arsanilic acid to the diet resulted in a marked reduction in the loin and introscope fat measurements, requires further investigation, particularly since there was no such effect when either of the two supplements was given separately.

The large increases in the concentration of Cu in the livers of the pigs given Cusupplemented diets in Expts 1 and 2 (no liver analyses were made in Expt 3) were within the range of those previously observed (Braude, 1965). The reductions in liver weight and liver Cu concentration by arsanilic acid do not appear to have been previously reported, although a similar reduction in liver weight has been frequently observed as a result of dietary supplementation with either Cu or antibiotics (Barber, Braude, Chamberlain & Mitchell, 1961), and it has been shown by several workers that the addition of 100-250 ppm zinc to a diet containing a supplement of 250 ppm Cu reduces the liver Cu concentration to an extent similar to that brought about by arsanilic acid in the present experiment (see Allen, Barber, Braude & Mitchell, 1958; Mills, 1968; Haranhan & O'Grady, 1968; Ritchie, Luecke, Baltzer, Miller, Ullrey & Hoefer, 1963). No determinations of liver arsenic concentration were made by us, but Hanson, Carpenter, Aunan & Ferrin (1955) reported a mean liver arsenic concentration (ppm As<sub>2</sub>O<sub>3</sub> in wet tissue) of 3·3 in pigs given 90 mg arsanilic acid/kg diet right through to the day of slaughter at about 90 kg live weight (as it was in the experiment reported here) and of 1.4 ppm when the arsanilic acid was withdrawn from

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