NUTRITIONAL REQUIREMENTS AND METABOLIC INHIBITION OF SOME ACARID MITES

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ABSTRACT: The nutritional requirements of two acarid mites *Tyrophagus putrescentiae* (Schrank) and *Caloglyphus berlesei* (Michael) have been elucidated. Metabolic inhibition was studied with possible view of a practical approach to nutritional control of these mites of stored or processed products. Malonic acid was inhibitory at 0.0001 percent in an axenic chemically defined diet. The example served here is the probable interference of malonate inhibition with several enzymes especially succinic dehydrogenase concerned with the citric acid cycle. Antagonists for such water soluble vitamins as Thiamine, folic acid, choline, riboflavin, and niacin were also studied and these antagonists offer possibilities as metabolic inhibitors for these acarids. Cholesterol, a required nutrient, can also be inhibited by the use of cholesteryl chloride. Less understood inhibition was demonstrated with the use of such antimicrobials as sodium propionate, 3,3-thiodipropionic acid and potassium sorbate.

The acarid mite, *Tyrophagus putrescentiae* (Schrank), is a cosmopolitan species found infesting many food products [1,2]. Our experience with this mite is that it is quite often a pest of processed foodstuffs such as cheeses, pet foods, and high protein livestock feeds. Another acarid species, *Caloglyphus berlesei* (Michael) may infest processed foods but only if they contain high moisture. This species may, in fact, be a pest more in production line situations where the foodstuffs from a production stream accumulate due to mechanical problems or breakdowns. Such pockets or deposits of foodstuffs, if wet, attract and support infestation of *C. berlesei*.

Both mites thrive well on the same artificial diet except for a difference in water content [3]. *T. putrescentiae* has a critical equilibrium activity of $a_w = 0.60$, while the water requirement for *C. berlesei* are much higher. Hence, the moisture content of the food and/or the relative humidity of the habitat become very important in the population dynamics of these species.

The application of nutritional principles, especially in the protection of processed foods, deserves critical consideration, because as Pratt *et al* [4] have pointed out, this avenue to control offers prospects of being a useful tactic. Food additives comprise a necessary fraction of the processed food components, and some of these may well be detrimental to an arthropod pest. We have, for example, during the course of our research in the

nutritional physiology of acarina observed that some materials added to artificial diets as antimycotic agents inhibited the development of mites as well. Because of the wide commercial application of such food preservatives as the salts of sorbic and propionic acids, it became our objective to investigate this aspect of food technology as a means of inhibiting the development of these acarid species.

It had previously been shown by Rodrigues [3] that short chain fatty acids were relatively effective in inhibiting the development of *T. putrescentiae* and *C. berlesei*. Also it was shown that caproate, caprylate, and caprate effectively inhibited *T. putrescentiae*. It was deemed important to study the effects of these methyl esters alone and in combination with potassium sorbate.

Perhaps even more fundamental than the above approach, however, is a more direct application of a nutritional control principle; i.e., the use of nutritional antimetabolites. It suffices to say that the application of an antagonist is to create abnormal nutrition and these processes in turn short circuit or impair efficient metabolism. Hence, another objective was to gain some information on the possible effect of some basic nutrient antagonists affecting these acarid species.

MATERIALS AND METHODS: Germfree procedures were used in handling the acarid mites. Axenic cultures of both species were started by surface sterilizing eggs with 2 percent formalin for 5 min. The eggs were rinsed in sterile distilled water and inoculated into 3-dram vials containing the test diet. The newly hatched larvae (20) were transferred to the test diet (5 replicates). Two such experiments were conducted for each treatment. The study on the combination of methyl esters and potassium sorbate utilized the casein (vitamin free)-wheat germ diet developed by Rodriguez for these 2 species [3]. In the propionate (calcium/sodium) and potassium sorbate study, the lipid fraction utilized in the chemically defined diet developed for *T. putrescentiae* by Rodriguez and Lasheen [5] was substituted for the wheat germ. The study on nutrient antagonist effects utilized also the chemically defined diet mentioned previously [5].

Visual counts were made by examining the vials under the dissecting microscope usually at 7, 14 and 28 days. The counts at 7 days were usually of immature forms only, while 14 and 28-day counts reflected all forms, i.e., larvae, nymphs, adults and eggs.

RESULTS AND DISCUSSION: When *T. putrescentiae* and *C. berlesei* were fed on a casein-wheat germ diet containing potassium sorbate and/or the methyl esters of fatty acids, populations (of adults, nymphs and larvae) were reduced from that of the control diet (Table 1). Inhibition of both species by potassium sorbate alone was greater at 0.5% than at 0.25%, especially after the developmental period of only 14 days. Among the methyl esters, when it was fed at 0.5%, methyl caproate ultimately (at 28 days) effected a 97.5% population reduction on *T. putrescentiae* followed by methyl caprate (92.2%)

and methyl caprylate (76.6%). Only the effect of methyl caprate, however, was manifested strongly at 14 days (89.8%). When fed at 0.5% in combination with potassium sorbate either at 0.25 or 0.5%, the same relative order of inhibition was noted, with methyl caprate again showing the strongest inhibition at 14 days. Total inhibition of *T. putrescentiae* occurred at 0.5% potassium sorbate combined with 0.5% methyl caprate.

TABLE 1. Percent reduction of *T. putrescentiae* and *C. berlesei* when fed potassium sorbate (KS) and fatty acid methyl ester (ME) combinations in casein-wheat germ diet.

Days	% KS / % ME	0 / 0.5	0.25 / 0	0.25 / 0.5	0.5 / 0	0.5 / 0.5
	T. putrescentiae					
	Caproate					
14		32.9	26.3	40.8	43.4	39.5
28		97.5	96.0	98.1	97.3	98.7
	Caprylate					
14		40.5	47.1	57.0	68.6	69.4
28		76.6	64.7	91.4	90.9	91.4
	Caprate					
14		89.8	69.3	94.0	88.4	93.0
28		92.2	90.6	99.0	97.0	100.0
	C. berlesei					
	Caproate					
14		41.0	82.6	92.6	88.0	82.9
28		52.0	98.8	99.2	99.2	99.4
	Caprylate					
14		72.8	85.3	86.7	99.3	99.6
28		48.3	99.5	99.6	100.0	100.0
	Caprate					
14		14.2	51.1	66.2	85.0	82.2
28		52.0	58.8	17.0	65.0	69.7

The methyl esters were less inhibitory to *C. berlesei* than to *T. putrescentiae;* when fed alone at 0.5% to this species, none of the methyl esters reduced the population by more than 52% after 28 days of development. In some populations of *C. berlesei*, greater than 98% reduction occurred on potassium sorbate alone; in such sensitive populations, little further inhibition would be possible from methyl ester-sorbate combinations.

On examining the population reductions at 14 days, there were some indications of partial additive effects of the methyl esters and the lower level of sorbate. For example, methyl caproate at 0.5% reduced *T. putrescentiae* by 32.9%, potassium sorbate at 0.25% by 26.3%; combined, a 40.8% reduction occurred. Methyl caprate at 0.5% reduced *C. berlesei* by 14.2%, potassium sorbate at 0.25% by 51.1%; combined, a 66.2% reduction occurred. Potentiation, or synergistic effects were not observed at the levels

of inhibitors used.

When either the calcium or sodium salt of propionic acid was fed to the two mite species alone or combined with potassium sorbate, the growth and development of these acarids was inhibited (Table II). *T. putrescentiae* populations were reduced more by calcium propionate than by sodium propionate at both 1% and 2% concentrations and at both the 14 and 28-day developmental times. *T. putrescentiae* was more sensitive than *C. berlesei* to 1% calcium propionate at 14 days, and *C. berlesei* was more sensitive than *T. putrescentiae* to both levels of sodium propionate at 14 days. However, at 28 days the inhibitory effects manifested against the two species by a given propionate were nearly equivalent, but stronger effects were shown by calcium propionate than by sodium propionate: at 1.0 and 2.0% CaP, *T. putrescentiae* were reduced by 98.2 and 99.3% and *C. berlesei* were reduced by 95.5 and 94.4%; while at 1.0 and 2.0% NaP, *T. putrescentiae* were reduced by 85.9 and 87.6% and *C. berlesei* were reduced by 86.6 and 93.9%; respectively.

TABLE II. Percent reduction of *T. putrescentiae* and *C. berlesei* when fed calcium or sodium propionate and potassium sorbate (KS) added to casein-lipid diet.

		Percent in diet							
Day	Propionate	1.0	2.0	0	1.0	2.0	0	1.0	2.0
	KS	0	0	0.5	0.5	0.5	1.0	1.0	1.0
	Calcium propionate								
	T. putrescentiae								
14		89.9	90.6	87.2	94.0	90.6	82.6	85.2	86.6
28		98.2	99.3	99.6	99.6	99.3	99.3	99.3	100.0
	C. berlesei								
14		66.4	91.4	72.1	93.0	91.0	93.0	92.6	93.8
28		95.5	94.4	99.7	99.5	100.0	99.6	99.8	100.0
	Sodium propionate								
	T. putrescentiae								
14		48.2	55.6	38.3	35.8	37.0	46.9	45.7	53.1
28		85.9	87.6	83.3	86.8	86.8	85.5	87.6	90.3
	C. berlesei								
14		69.3	80.2	93.9	96.9	98.0	98.2	97.2	98.4
28		86.6	93.9	93.3	99.4	99.7	99.2	99.6	99.9

In the propionate studies, as in the methyl ester studies, the inhibition by potassium sorbate or calcium propionate alone was sufficiently severe that little further inhibition could be brought about by feeding a combination. The term "total inhibition" imples not only 100% population reduction to 0 but also the complete suppression of oviposition which unless inhibited started prior to the 14-day reading. From the practical standpoint, a lasting inhibition of a population occurs when the numbers of adults, nymphs and larvae are largely reduced to a few hardy survivors but no oviposition takes place. Oviposition was

evaluated in the sorbate-propinate studies but is not shown in the Tables. The oviposition results could not be so directly correlated with percentage reduction of population that one could assume that above a particular percentage no oviposition would occur. For example, live forms of *T. putrescentiae* were not inhibited by more than 90.3% in the sodium propionate study, yet oviposition was completely suppressed by all dietary additions except 1.0% NaP, 0% KS. On the other hand, in the same study *C. berlesei* populations were able to produce rather large numbers of eggs even at 28 days on the 1% and 2% NaP, 0% KS, and 0% NaP, 0.5% KS treatments, as well as a few eggs on all the other treatments except 2.0% NaP, 1.0% KS.

Nutrient antagonists incorporated into *T. putrescentiae* diets effected population reductions of varying degrees (Table III).

TABLE III. Effect of nutrient antagonists on *T. putrescentiae* development.

Antagonist Fed	% Concn. in diet	Nutrient inhibited	% Population reduction from control diet 2 weeks	4 weeks
Malonate[a]	0.0001	Succinate	77.1	90.5
	0.0010		91.7	98.4
Pyrithiamine[b]	0.0015	Thiamine	0	0
	0.0150		46.4	81.4
Quinacrine[b]	0.002	Riboflavin	20.7	0
	0.020		43.7	40.4
	2.000		32.2	78.0
Picolinic acid[a]	0.0018	Niacin	61.4	57.9
	0.0180		97.6	95.2
Aminopterin[b]	0.002	Folic Acid	42.4	———
	0.020		65.4	———
2-Amino-2-methyl 1-propanol[a]	0.03	Choline	29.8	12.2
	0.30		26.3	86.9
	3.00		28.1	100.0
Cholesteryl chloride[a]	1.0	Cholesterol	———	77.5

[a] Chemically defined diet (Rodriguez and Lasheen, 1971)
[b] Casein-lipid diet (Rodriguez, 1972)
[c] Reading after 3 generations

The classic substrate inhibitor, malonate, though not strictly speaking a nutrient antagonist, has been included in Table III for convenience. As a competitive inhibitor of the succinate oxidase enzyme complex, it interferes with the TCA cycle and ATP production and is thus inhibitory to animal systems generally [6]. Its theoretical application as a mite inhibitor in foods would depend on finding a level that would suppress mite growth and development while not causing deleterious effects to the consumer. A

concentration of 0.0001% in the *T. putrescentiae* diet resulted in 90.5% reduction in the population at 4 weeks (Table III).

Antivitamins have been useful in certain studies in nutritional physiology [7]. In our limited study with this aspect of the problem the analogues of the water-soluble vitamins were added to the diets at two or three levels differing by a factor of 10 and giving a range of levels below, equal to and above the level of the corresponding vitamin in the control diet. Effective reduction of populations (78.0% and above) were achieved at 4 weeks by: 0.0150% pyrithiamine; 2.000% quinacrine; 0.0180% picolinic acid; 0.30 and 3.00% 2-amino-2-methyl-1-propanol.

A cholesterol analogue, cholesteryl chloride, at a level of 1.0% reduced the population by 77.5% after a long time period equivalent to 3 full generations. The apparent requirements for cholesterol by these acarids, although essential, are not easily manifested in suppression of mite numbers.

In a previous publication [3] it was indicated that short-chain fatty acids, e.g., propionic, butynic, caproic and selected methyl esters were effective inhibitors of *T. putrescentiae* when incorporated into dog food. The present work extends these findings to include the effects of the calcium and sodium salts of propionic acid and potassium sorbate on *T. putrescentiae* and on *C. berlesei* as well.

The actual mechanisms of the inhibitory effects of such dietary additives as sorbate and the propionates have not been established. These compounds are generally regarded as safe for mammalian consumption and are regularly added to human and pet foods as antimycotics. An immediate question that arises is whether they suppress mite populations by eliminating beneficial microorganisms associated with the mites. The axenic methodology of the dietary studies generally precludes this possibility. The antimicrobials streptomycin and methyl-p-hydroxybenzoate are components of the mite diets used in our axenic studies [3], and their presence in the control diets did not inhibit the mite populations. Since the mites were placed on the diets as sterilized eggs, symbionts could have been transferred only by trans-ovarial transmission. To our knowledge, this phenomenon has never been demonstrated in the acarines.

A mechanism for the metabolism of propionate in animal tissues was demonstrated by Ochoa and co-workers [8]. Propionate is enzymatically converted to propionyl CoA which is carboxylated to methyl-malonyl CoA. This compound is then isomerized to succinyl CoA, entering as succinate into the TCA cycle.

It is not known whether this pathway is operative in acarines and propionate is thus metabolized. Further research aimed at elucidating the nature of the inhibitory action of propionate on acarines would be profitable.

CONCLUSIONS: The technology currently applied in processed foods where food additives include preservatives of the salts of propionic acid and potassium sorbate as protection against mold growth, now

should harmonize that application with that of inhibiting growth and development of the acarid mites, especially *T. putrescentiae*. The methyl esters of caproate, caprylate and caprate may also have practical application. They were effective inhibitors of both *T. putrescentiae* and *C. berlesei* when used either singly or in combination with potassium sorbate. Combinations of any of the inhibitors did not trigger apparent potentiation or synergism, and effects remained additive and dosage related. A practical application of the classical antagonists or antimetabolites such as malonate and antivitamins would depend on finding a correct level at which the antagonist would be creating a nutrient deficiency for the mite species but not for the intended consumer of the foodstuff.

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