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THE INHERITANCE OF A-PYRIDINE NUCLEOSIDASE IN BULL SEMEN

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SUMMARY

Semen samples from each of 300 dairy and 41 beef sires were assayed for pyridine nucleosidase activity. Among the dairy sires only 16% produced semen free of this enzyme, whereas the comparable figure among the beef sires was 54%. The highest enzyme concentration for a dairy sire was 1573 units/ml of semen compared with 630 units in two beef sires.

The distribution of enzyme concentrations among the dairy sires showed groups of animals at intervals of approximately 300 units/ml. This result suggested that the concentration of seminal pyridine nucleosidase was simply inherited and may involve the additive effects of four alleles at two loci. This hypothesis was supported by grouping results for 28 sires each with from 2 to 17 sons.

The measurement of seminal components, particularly proteins and enzymes produced by accessory sex glands could be a useful means of studying gene expression in cattle.

INTRODUCTION

Nicotinamide nucleotide coenzymes have an important role in cell metabolism. In motile sperm the ratio between oxidized nicotinamide adenine dinucleotide (NAD^+) and the amount of its reduced form (NADH) is regulated in a rapid and highly sensitive manner by energy yielding processes originating in the extracellular environment. Therefore exogenous substrates either secreted by the male or female reproductive tract, or incorporated in artificial semen diluents, can influence the metabolic behaviour of sperm (Brookes and Mann, 1971).

In several domestic mammals the concentrations of NAD measured in epididymal sperm are similar, but there are major differences in NAD levels in ejaculated sperm between species (Macmillan *et al.*, 1975a). These differences primarily arise because of the presence or absence of a pyridine nucleosidase which is secreted at least in the bull by the seminal vesicles (Leone and Bonaduce, 1959). This enzyme rapidly destroys over 60% of the NAD bound to epididymal sperm within minutes of ejaculation (Bisticchi *et al.*, 1968).

The initial report that some bulls do not produce this seminal constituent was made by Hathaway and Chamberlain (1970). When Macmillan *et al.* (1975a) studied differences between sires in the concentration of pyridine nucleosidase, they observed groups of sires at intervals of 300 units of enzyme per ml of semen. This paper examines results from dairy and beef sires with a view to determining the possible mode of inheritance of enzyme concentration.

MATERIALS AND METHODS

Semen was collected by artificial vagina from 300 dairy sires (154 Jerseys, 136 Friesians and 10 Ayrshires) and 41 beef sires of five different breeds. A 0.05 ml sample of semen from each ejaculate was added to 5 ml of cold 2% tri-sodium citrate. The diluted samples were stored at -10°C until they were assayed. Following thawing and filtration (Whatman No. 1), 1 ml of a diluted sample was warmed to 37°C when $1\ \mu$ mole of NAD (0.1 ml volume) was added and the mixture shaken and incubated for a further 10 min. Enzyme activity was terminated by the addition of 9 ml of 1M sodium cyanide solution. After cooling, the NAD concentration was determined spectrophotometrically at 327 nm (Ciotti and Kaplan, 1957). With each sample in which more than $0.5\ \mu$ moles of NAD was hydrolysed, the assay was repeated using 0.5, 0.25 or 0.1 ml of the semen filtrate. Pyridine nucleosidase concentration was expressed in units per ml of semen with each unit representing the amount of enzyme that would hydrolyse $1\ \mu$ mole of NAD in 1 h (Zatman *et al.*, 1953).

Details relating to the stability of the enzyme during storage and the repeatability of the assay have been reported elsewhere (Macmillan *et al.*, 1975a).

RESULTS AND DISCUSSION

Like other seminal components, the concentration of pyridine nucleosidase will vary between ejaculates from a single sire. This variation is greatest among sires producing highest concentrations of this enzyme. Only a single semen sample from most of the 341 sires was assayed. However, sampling up to 13 ejaculates from each of five sires whose semen contained no enzyme showed that the absence of pyridine nucleosidase was neither a phenomenon of chance nor the consequence of enzyme inhibition (Macmillan *et al.*, 1975a).

When results for the dairy sires were classified into groups of 100 enzyme units, the distribution pattern showed several peaks and troughs with the peaks occurring at multiples of approximately 300 enzyme units (Fig. 1). As the distribution patterns in enzyme concentration among Jersey and Friesian sires were similar, the results for these sires have been combined. Enzyme concentrations for those bulls recorded within a trough tended towards the lower or higher extremity of the particular 100 enzyme unit range.

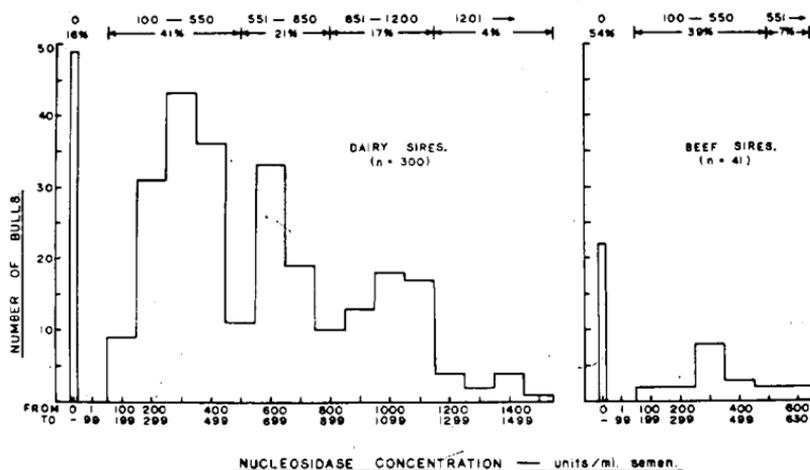


Fig. 1: Nucleosidase concentration in the semen of dairy and beef sires.

Among the dairy sires, 16% produced no seminal pyridine nucleosidase and the highest concentration was 1573 units/ml of semen. In marked contrast, 54% of the beef sires produced semen free of this enzyme and the highest concentration was 630 units/ml (Fig. 1). Only 5 of 17 Hereford sires produced pyridine nucleosidase in their semen. These results indicate that there are significant differences between breeds in the occurrence and range of concentrations of this seminal constituent. Further sampling from beef sires may show a difference between beef and dairy sires.

Among the dairy sires, the presence of four distinct peaks may reflect a relatively simple form of inheritance for enzyme concentration. These peaks would include sires producing semen with enzyme concentrations of 0, 100 to 550, 551 to 850 or 851 to 1200 units/ml. If the 11 sires whose semen contained more than

1200 units of enzyme per ml, also represented another group, then there would be five concentration groupings (Table 1). Each group could be produced by the interactions between two separate alleles at two loci. At each locus, one allele would facilitate production of 300 enzyme units with this effect being additive (Table 2). In this case only one locus may be involved with the production of pyridine nucleosidase in semen from beef sires (Fig. 1).

TABLE 1: DISTRIBUTION OF SEMINAL NUCLEOSIDASE CONCENTRATIONS AMONG SONS OF SIRES WITH VARIED CONCENTRATIONS

Sires		Sons				
Enzyme Conc. (units/ml)	No.	Enzyme Concentrations (units/ml)				
		0	100-550	551-850	851-1200	>1200
0	3	8 (44%)	7 (39%)	2 (11%)	1 ¹ (6%)	0
100-550	14	24 (22%)	40 (37%)	23 (21%)	19 (18%)	2 ¹ (2%)
551-850	2	2 (8%)	11 (44%)	6 (24%)	5 (20%)	1 (4%)
851-1200	6	1 ¹ (3%)	16 (42%)	10 (26%)	8 (21%)	3 (8%)
1200	3	0	7 ¹ (27%)	12 (41%)	5 (19%)	2 (8%)

¹ Possibly mis-identified progeny.

The results for the 300 dairy sires were insufficient to determine gene frequencies accurately, but among the 290 Jersey and Friesian bulls were data for 28 sires which each had records for 2 to 17 sons. When sires and sons were each classified into one of five groups based on enzyme concentration, it was apparent that pyridine nucleosidase levels were inherited (Table 1). Three sires produced semen free of pyridine nucleosidase and 8 of their 18 sons (44%) also produced semen free of this enzyme. All 26 sons sired by three sires from the highest enzyme concentration group produced seminal pyridine nucleosidase.

Within the data recorded in Table 1 are 11 sons which produced semen containing enzyme concentrations which do not fall within the range predicted by the hypothesis of inheritance involving two alleles at two loci. Although these could arise because of errors in

sampling, assaying or recording, it is also possible that some of the sons may not be progeny of the recorded sire. Seminal plasma contains a variety of proteins and enzymes (Mann, 1964). Measuring the concentration and structural differences in these seminal constituents from groups of progeny could be a convenient technique for studying gene expression and forms of inheritance. The results could also have applications similar to blood typing.

TABLE 2: POSSIBLE FORM OF INHERITANCE FOR THE CONCENTRATION OF PYRIDINE NUCLEOSIDASE IN SEMEN FROM DAIRY SIREs

(Each large letter is associated with the production of approximately 300 units of enzyme/ml)

Allelic Configuration	Enzyme Concentration (units/ml)				
	0	100-550	550-850	850-1200	>1200
aabb	Aabb	AAbb	AABb	AABB	
	aaBb	aaBB	AaBB		
		AaBb			

Some of these inherited constituents in seminal plasma may influence sperm metabolism and affect post-insemination livability to produce fertility differences between sires (Macmillan, 1973). If some of the components producing these effects were simply inherited, the high heritability of conception rate (0.54) recorded by Shannon and Searle (1962) would be a predictable result.

The presence or absence of the seminal pyridine nucleosidase does not influence fertility when semen is processed in Caprogen containing catalase (Macmillan *et al.*, 1975b). Nonetheless, the presence of an enzyme in seminal plasma which destroys a cell-bound co-enzyme which is essential for many metabolic processes may initially appear as another enigma together with the toxic component of seminal plasma (Shannon *et al.*, 1974) and the dead sperm factor (Shannon and Curson, 1972; Macmillan *et al.*, 1972). The high levels of NAD in epididymal sperm may influence respiration prior to ejaculation as Robinson (1974) reported that the addition of NAD to mitochondrial preparations from ejaculated sperm inhibited respiration.

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