STUDY OF THE GLUCOSE, ELECTROLYTES, ENZYMES AND NITROGEN COMPONENTS OF FOWL SEMINAL PLASMA*

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Summary. This study was undertaken to determine some of the biochemical components of fowl seminal plasma. The semen used in this investigation was collected by artificial ejaculation of 18-month-old White Plymouth Rock males.

Glucose concentration was 74.2 ± 7.8 mg glucose/100 ml of cock seminal plasma. Several enzyme analyses were conducted, using incubation, colorimetry and titration techniques. The enzyme concentrations found were as follows: acid phosphatase, 106 ± 6.5 sjr units; alkaline phosphatase, 6.7 ± 0.83 sjr units; glutamic pyruvic transaminase, 12.8 ± 1.4 GPT units; glutamic oxalic transaminase, 560 ± 69.6 GOT units; lactic dehydrogenase, 4610 ± 251 LDH units and leucine amino peptidase, 2474 ± 436 LAP units. Lipase was not found to be present under the conditions of these analyses. Nitrogen concentration as determined by micro-Kjeldahl method averaged 180 mg/100 ml of fowl seminal plasma. Free amino acids found were glutamic acid, glycine, arginine, alanine, serine and methionine. Bound amino acids found were α - or β -alanine, serine, threonine, valine, methionine, tryptophan, leucine (isoleucine, norleucine or leucine) aspartic and glutamic acids, arginine, lysine, histidine, cystine, phenylalanine tyrosine, proline and several unidentified spots.

INTRODUCTION

Although reproductive failures are a source of concern to poultrymen, the biochemical composition of fowl seminal plasma has received little attention. Harris & Wilcox (1962) found glucose and fructose were utilized to a much greater extent than other carbohydrates when added to chicken semen. Glucose concentration was much higher than that of other carbohydrates found by DeMuelenaere & Quicke (1959), Yoshida & Masuda (1962), Nishiyama (1957) and Mann (1954). Alkaline and acid phosphatase concentrations were determined by Bell & Lake (1962) and Wilcox (1961). Other enzymes of fowl seminal plasma analysed in this study have not been previously reported in the

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literature. Electrolytes of fowl seminal plasma were studied by Lake, Butler, McCallum & MacIntyre (1958) and Takeda (1959). Nitrogen compounds have been studied by Pytasz & Klymiuk (1961) and Wales, Scott & White (1961). The amino acids were studied by Chubb & Cooper (1962), Goldberg, Pence & Norman (1961) and Yoshida & Masuda (1962).

The object of this experiment was to identify some of the biochemical components of fowl seminal plasma. These components include glucose, nitrogen, some of the electrolytes, enzymes and identification of some of the amino acids, both bound and free.

METHODS

Semen from each of six White Plymouth Rock males, 18 months old, was collected three times weekly by the method of Burrows & Quinn (1937), with at least 1 day between collections. The volumes were determined by using a 1 ml tuberculin syringe. Spermatozoa count employed the haemocytometer technique, and motility (percentage showing movement) and vigour as used by Boone & Huston (1963). These measurements were made at room temperature, approximately 25° C, between 30 min and 1 hr after collection. Seminal plasma was then separated from the spermatozoa by centrifugation at approximately 2000 *g* for 5 min and decantation of the seminal plasma to another tube. The centrifugation and decantation were repeated to insure removal of all spermatozoa. Samples were frozen at 0° F. The three seminal plasma samples from each male were pooled weekly. All samples were kept well below room temperature prior to the analyses by immersing them in an ice bath. Glucose analysis was made by the method of Huffman (1937) as modified for use on the Technicon auto analyser (1960).

Acid and alkaline phosphatases were analysed by the method of Shinowara, Jones & Reinhart (1942). Each unit was equal to the amount of phosphatase that liberates 1 mg of inorganic phosphorus from β -glycerolphosphate in 1 hr under conditions of test. Lactic dehydrogenase was determined by the method of Cabaud & Wroblewski (1958). Each unit is equivalent to the amount of enzyme that would cause a decrease in optical density of 0.001/min in a reaction mix of 3 ml of sample. Lipase determinations employed the method of Tietz, Borden & Stepleton (1959). Lipase units are equal to the number of ml of 0.05 *N*-sodium hydroxide required to neutralize the free fatty acid liberated by the lipase in the sample during a 6 hr incubation period. Leucine amino peptidase (LAP) was determined by Sigma's (1962) modification of the method of Goldbarg & Rutenburg (1958). One LAP unit is equivalent to 0.0417 μ g β -naphthylamine liberated in 1 hr at 37° C. Glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) transaminases were determined by the method of Reitman & Frankel (1957). Each GOT and GPT unit is equivalent to 1 μ g of oxaloacetic acid and pyruvate respectively produced under conditions of the test.

Electrolyte concentrations, except chloride, were determined using a flame photometer. Chloride concentration required the use of a chloridimeter. Nitrogen concentration of composite samples in duplicate were determined by the micro-Kjeldahl (A.O.A.C., 1955) method.

Both bound and free amino acids of the fowl seminal plasma are identified

by the method of Chubb & Cooper (1962). This required the use of two dimensional chromatography (Block & Weiss, 1956). n-Butanol, glacial acetic acid and water (4:1:1) was the first solvent and pyridine and water (4:1) was the second.

An analysis of variance (Steel & Torrie, 1960) was made for differences among males. Estimates of correlation of glucose, enzymes and electrolytes with percentages of motility, sperm vigour and number of sperm were made. Standard errors of the mean follow the average glucose, electrolyte and enzyme concentrations.

RESULTS AND DISCUSSION

Carbohydrate and enzyme analyses of semen indicate the metabolism of the sperm to be concerned mainly with the glycolytic scheme (Mann, 1954). He found glucose to be the major carbohydrate present with small amounts of fructose and trace amounts of others. In this study, 74.2 ± 7.8 mg of glucose (Table 1) per 100 ml of seminal plasma were found. Mann (1954) found

TABLE 1
AVERAGE GLUCOSE CONCENTRATION IN THE
SEMINAL PLASMA OF THE FOWL BASED ON
FOUR ANALYSES PER MALE*

Male No.	mg/100 ml
4584	88.00 ± 11.54
4622	56.00 ± 13.33
4639	88.00 ± 25.10
4108	90.00 ± 16.33
4544	58.67 ± 13.33
Weighted average	74.15 ± 7.80

* $\pm$ Standard error of the mean.

7.7 to 81 mg of glucose/100 ml of fowl semen. Harris & Wilcox (1962) found that glucose was utilized as a source of spermatozoa energy when added to diluted semen as measured by changes in pH, carbohydrate disappearance and lactic acid production.

Some phosphatases, both acid and alkaline, are involved in the hydrolysis of carbohydrates. Acid phosphatase, in this study, averaged 106.3 ± 6.5 sjr units (Table 2).

Alkaline phosphatase averaged 6.7 ± 0.8 sjr units/ml. Bell & Lake (1962) also found the acid phosphatase values to be much higher than the alkaline phosphatase.

No previous analyses of transaminase, peptidase, lactic dehydrogenase or lipase concentrations in fowl semen or seminal plasma were found in the literature. The average concentration of glutamic pyruvic transaminase (Table 2) was 12.8 ± 1.4 units. Lactic dehydrogenase averaged 4610 ± 251 units/ml of seminal plasma (Table 2). This enzyme catalyses the following reaction: pyruvic acid + DPNH $\rightleftharpoons$ lactic acid + DPN (White, Handler, Smith

TABLE 2
AVERAGE CONCENTRATION OF ENZYMES IN SEMINAL PLASMA OF THE FOWL BASED ON FOUR ANALYSES* (MG/ML)

Male No.	Acid phosphatase (SJR units)	Alkaline phosphatase (SJR units)	Glutamic pyruvic transaminase (GPT units)	Glutamic oxalacetic transaminase (GOT units)	Lactic dehydrogenase (LDH units)	Leucine amino peptidase (LAP units)
4584	103.50 ± 14.98	7.40 ± 1.67	13.25 ± 3.88	771.50 ± 125.66	4125.00 ± 519.98	3076.50 ± 1604.4
4622	92.60 ± 17.34	10.30 ± 2.37	11.50 ± 3.88	720.00 ± 125.66	4954.33 ± 611.24	3809.00 ± 3006.8
4639	98.78 ± 14.98	5.87 ± 1.93	15.75 ± 3.88	500.00 ± 125.66	3635.00 ± 519.98	4966.50 ± 3682.7
4108	89.28 ± 14.98	3.70 ± 1.93	17.50 ± 3.88	385.00 ± 125.66	2715.00 ± 519.98	3625.00 ± 3006.8
4544	122.15 ± 14.98	5.93 ± 1.67	10.00 ± 3.88	370.00 ± 125.66	5345.00 ± 519.98	4001.33 ± 3006.8
4225	128.30 ± 14.98	8.10 ± 1.93	9.00 ± 3.88	610.00 ± 125.66	9340.00 ± 748.77	1257.50 ± 3682.7
Weighted average	106.34 ± 6.54	6.68 ± 0.83	12.83 ± 1.35	560.42 ± 69.61	4610.62 ± 251.41	3474.12 ± 435.9

* ± Standard error of the mean

& Stetten, 1959). This is an important reaction involved in anaerobic energy metabolism. The average concentration of glutamic oxaloacetic transaminase, also known as glutamic aspartic transaminase, was 560 ± 69.6 units. This enzyme catalyses the following reaction: aspartic + α keto glutaric acid $\rightleftharpoons$ oxaloacetic + glutamic acid. Aspartic and oxaloacetic acids are parts of the Krebs cycle (White *et al.*, 1959). Leucine amino peptidase averaged 3474 ± 436 units (Table 2). This enzyme hydrolyses peptide chains at the leucine amino group. Lipase is an enzyme catalysing the breakdown of fats to fatty acids and glycerol. This enzyme was not found in the two samples of seminal plasma examined. This indicates that fat metabolism may be very low or absent in spermatozoa.

Electrolytes play an important role in homeostasis. Both osmotic pressure and pH are greatly affected when these electrolytes are not in correct proportion or balance. The ions of sodium, potassium, chloride, calcium, bicarbonate,

TABLE 3

AVERAGE CONCENTRATION OF ELECTROLYTES IN SEMINAL PLASMA OF THE FOWL BASED ON FOUR ANALYSES* (MG/100 ML)

Male No.	Na	Ca	K	Cl
4584	385.25 ± 25.77	6.80 ± 0.97	45.63 ± 8.61	553.25 ± 32.9
4622	334.08 ± 25.77	6.50 ± 0.97	54.20 ± 8.61	369.50 ± 46.6
4639	331.78 ± 25.77	7.00 ± 0.97	43.08 ± 8.61	563.67 ± 38.1
4108	381.23 ± 25.77	8.50 ± 0.97	38.43 ± 8.61	498.25 ± 32.9
4544	276.58 ± 25.77	5.75 ± 0.97	49.70 ± 8.61	478.75 ± 32.9
4225	310.50 ± 29.76	7.87 ± 1.11	35.63 ± 9.96	401.00 ± 65.9
Weighted average	337.70 ± 17.19	7.03 ± 0.40	44.83 ± 2.76	497.33 ± 27.7

* $\pm$ Standard error of mean.

magnesium, phosphate and sulphate are involved in homeostasis of spermatozoa as indicated by Mann (1954). Calcium averaged 7.0 ± 0.4 mg/100 ml (Table 3). This concentration was lower than the 8.4 mg of calcium/100 ml of fowl seminal plasma reported by Takeda (1959) and 9 mg/100 ml reported by Lake *et al.* (1958). Potassium concentration averaged 44.8 ± 2.8 mg/100 ml (Table 3). This was within the range (39 to 49 mg/100 ml) found by Lake *et al.* (1958). Takeda (1959), however, found 29.7 mg/100 ml. Sodium averaged 338 ± 17 mg/100 ml (Table 3), which agreed closely with both Lake *et al.* (1958) and Takeda (1959) who found 379 and 332.5 to 346 mg/100 ml respectively. Chloride averaged 497 ± 27.7 mg/100 ml (Table 3). This concentration was much higher than that reported by Lake *et al.* (1958) who found 197 to 212 mg/100 ml. Concentrations of the various electrolytes and other components and volumes of semen may vary, since all investigators did not employ the same ejaculation technique and breeds of fowl or analytical methods. In these studies the volume of seminal fluid/ejaculate averaged 2.07 ml, while Sturkie (1954) reported an average of 0.75 ml/ejaculate.

Total nitrogen (Table 4) as determined by micro-Kjeldahl method averaged 180 mg/100 ml of seminal plasma. Pytasz & Klymiuk (1961) using paper

electrophoresis found 593 mg of nitrogen/100 ml. The differences may, in part, be due to presence of urates (uric acid) in the latter, a dilution factor in the former due to collection technique, or the difference in analytical methods used.

Protein components were studied by amino acid analyses. In this study, α - or β -alanine, serine, threonine, valine, methionine, tryptophan, leucine (isoleucine, norleucine, or leucine), aspartic and glutamic acids, arginine, lysine, histidine, cystine, phenylalanine, tyrosine, proline and several unidentified spots were noted in the bound amino acid fraction. In addition to these, Chubb & Cooper (1962) found glycine, glutamine, taurine, asparagine, citrulline and α -amino-butyric acid. Free amino acids found were glutamic acid, α - or β -alanine, glycine, serine, arginine, methionine, leucine and several unidentified spots. Chubb & Cooper (1962) found 80 % glutamic acid and some α -alanine, aspartic acid, glycine and serine. Goldberg *et al.* (1961), found 79 % glutamic acid, along with glycine, arginine, alanine and trace amounts of other free amino acids.

TABLE 4
NITROGEN CONCENTRATION OF FOWL
SEMINAL PLASMA

<i>Week</i>	<i>Nitrogen</i> (mg/100 ml)
1	218
2	127
3	184
4	162
Average	180

Based on micro-Kjeldahl determination,
using composite samples run in duplicate.

This large amount of glutamic acid would be expected since, as previously noted, glutamic acid is an end product of glutamic-oxaloacetic and pyruvic transaminase reactions.

Since glucose, enzymes and electrolytes play an important part in the metabolism of the spermatozoa, the correlations of these biochemical components with the percentage of motile spermatozoa, spermatozoal vigour and number of spermatozoa were estimated. It is apparent from Tables 5 and 6 that the correlations were significant in only four cases. Table 5 shows a highly significant positive correlation between the amount of lactic dehydrogenase/spermatozoa and the percentage of motile spermatozoa. This enzyme may play an important role in the spermatozoon's anaerobic energy metabolism during the quiescent period of the spermatozoon's life, and thus the amount of lactic dehydrogenase would increase as the number of active spermatozoa increases.

The speed (vigour) with which the spermatozoon moved across the microscopic field apparently bore no significant relationship to the quantity of any of the biochemical components studied (Table 5).

Table 6 shows a highly significant positive correlation between the amount of acid phosphatase and the number of sperm. The highly significant negative

correlation between numbers of spermatozoa and glucose suggests the utilization of glucose by the spermatozoa. A significant negative correlation between the concentration of chloride in seminal plasma and the number of spermatozoa was found. This suggests the possibility of the chloride ions shifting into the spermatozoa while carbon dioxide is lost.

TABLE 5

CORRELATION COEFFICIENT OF THE BIOCHEMICAL COMPONENTS OF SEMINAL PLASMA/SPERM WITH PER CENT MOTILE SPERM AND SPERM VIGOUR IN FOWL SEMEN

<i>Biochemical component</i>	<i>Motile sperm (%) r</i>	<i>Vigour r</i>
Glucose	-0.4412	-0.3130
Acid phosphatase	-0.1692	-0.1602
Alkaline phosphatase	-0.1956	-0.3200
Leucine aminopeptidase	-0.0088	-0.2385
Lactic dehydrogenase	0.5515*	0.2012
Glutamic oxaloacetic transaminase	-0.1911	-0.1495
Glutamic pyruvic transaminase	0.1630	0.1411
Sodium	-0.1207	-0.0429
Calcium	-0.1296	0.1516
Potassium	-0.1794	-0.1515
Chloride	-0.1581	-0.0843

* Significant at the 0.01 level.

TABLE 6

CORRELATION COEFFICIENT OF THE BIOCHEMICAL COMPONENTS/ML OF SEMINAL PLASMA WITH NUMBER OF SPERMATOZOA/ML IN FOWL SEMEN

<i>Biochemical component</i>	<i>r</i>
Glucose	-0.7236†
Acid phosphatase	0.6346†
Alkaline phosphatase	-0.0741
Leucine aminopeptidase	-0.3113
Lactic dehydrogenase	0.3599
Glutamic oxaloacetic transaminase	0.0472
Glutamic pyruvic transaminase	-0.1977
Sodium	-0.3020
Calcium	-0.1413
Potassium	0.1596
Chloride	-0.5235*

* Significant at the 0.05 level.

† Significant at the 0.01 level.

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