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Effects of Cornstarch and Dextrose on Oysters¹

BY

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INTRODUCTION

THE GROWTH AND FATTENING of the American oyster, *Crassostrea virginica* (Gmelin, 1791), has long been of interest to oyster biologists since the market value of oysters is directly related to the quantity and quality of "meat" obtained per bushel (= 35.2 L) of harvested oysters. Much of the early work done on the growth and fattening of oysters attempted to demonstrate the superior nutritional value of specific planktonic organisms and detritus (MOORE & POPE, 1910; MARTIN, 1923, 1927a, 1927b, 1928; GAVARD, 1927; NELSON, 1947). MITCHELL (1917) was the first to study the effects of carbohydrates of known composition on oysters. He reported that oysters held in standing seawater containing 0.25% glucose had higher glycogen levels than control oysters. YONGE (1928) showed that oysters were capable of removing dissolved carbohydrates from seawater. GILLESPIE, INGLE & HAVENS (1964) demonstrated that oysters receiving only dextrose at 30 mg/L lived an average of 68.2 days longer than starved oysters.

NELSON (1934) was the first to investigate effects of particulate carbohydrates on oysters. Although his report did not present quantitative data, he stated that only cornstarch was "successful." More recent investigators have studied the effects of particulate and dissolved carbohydrates on the glycogen content, tissue weight and shell size of oysters. HAVEN (1965) demonstrated that oysters receiving wheatflour or cornstarch at concentrations of 2 mg/L would, at certain seasons, have dry tissue weights significantly greater than controls receiving only flows of natural river waters. His results of supplemental feeding with dextrose were not definitive, and dextrose concentrations as high as 34 mg/L were required to produce a significant influence on dry tissue weight. These studies were corroborated by GILLESPIE, INGLE & HAVENS (1966). KUWATARI & NISHII (1967) showed that rice powder

added to the diet of the pearl oyster resulted in a higher tissue weight. In a more recent study, CASTELL & TRIDER (1974) demonstrated the nutritional value of carbohydrates, lipids and other dietary substances of known composition in the diet of oysters.

Previous authors have suggested that stored glycogen is a major factor influencing the size and quality of oyster tissue and have shown that the level of stored glycogen on a dry weight basis varies seasonally with a minimum of about 3% in late summer and a maximum of about 24% in early spring and late fall (ENGLE, 1950; HOPKINS, MACKIN & MENZEL, 1953). As a consequence of this seasonal change in glycogen, effects of supplements added at various seasons might vary.

There were three purposes to this study. The first was to define the minimum quantity of particulate carbohydrate (in the form of partially hydrolyzed cornstarch) necessary to produce a measurable increase in glycogen content, tissue weight, shell height, underwater shell weight and total volume of oysters. The second was to determine if the effect of starch varied with season. The third was to eliminate some of the confusion concerning the uptake and utilization of dextrose. That is, does supplemental feeding with low levels of dextrose result in statistically significant increases in glycogen content, tissue weight, shell height, underwater shell weight and total volume. Also, are these increases, if they occur, statistically comparable to those produced by cornstarch. In order to answer these questions oysters were offered dextrose in two forms: 1) as a solution; and 2) mixed with clay. The rationale behind mixing dextrose with clay was that soluble sugars are known to be adsorbed on clay particles similar to those present in marine waters (BADER, 1962). It was theorized, therefore, that if oysters were offered clay particles coated with dextrose they would ingest the coated particle and the dextrose would be stripped from the particle and assimilated. The digestive diverticula of oysters are known to be quite acidic (GALTSOFF, 1964), a situation which would favor this process.

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MATERIALS AND METHODS

Four experiments were conducted during the summer and fall of 1966 and the spring of 1967 using different concentrations of cornstarch, dextrose and clay. The types of supplements added, size and treatment of experimental groups, sources of oysters, size, meat measurements and statistical treatment of data are presented in this section and in Table 1.

Oysters for all studies were obtained from Horsehead Bar in the James River, Virginia, an area free of known oyster diseases (Andrews, personal communication). Experimental oysters were selected for uniformity (40 to 45 mm in shell height), freed of attached fouling, randomly separated into groups of 20 and numbered with an enamel paint. Each group of 20 oysters became a "unit" which was subject to various experimental conditions.

One day prior to the start of an experiment, the following measurements were taken on individual oysters in all groups: 1) underwater shell weight to the nearest 0.01 g by the method of ANDREWS (1961); 2) shell height to the nearest 0.1 mm with vernier calipers; and 3) total volume to the nearest 0.1 cc by water displacement. In addition, one group of 20 oysters was sacrificed on the first day of each experiment to determine the following measurements: 1) wet tissue weights to the nearest 0.01 g; and 2) glycogen levels estimated to the nearest 0.01% of the wet tissue weight. Wet tissue weights were recorded after the tissues had drained for 1 minute on a fine mesh, metal grid. Glycogen was extracted by the method outlined by CALDERWOOD & ARMSTRONG (1941) with the exception that the precipitated glycogen, rather than being collected on filter paper, was centrifuged down and the supernatant discarded (Armstrong, unpublished). Glyco-

Table 1

Specific Details of Experiments I, II, III and IV Showing Dates, Water Temperatures, Salinities and Treatments.

Exp. No.	Date	Temp. Range and (mean) C	Salinity Range and (mean) ‰	Treatment
I	7/ 7/66-13/ 8/66	27.9-29.1 (25.6) Max. Summer Temp.	20.4-22.4 (21.6)	1. Cornst.—2 mg/L 2. Dext.—2 mg/L 3. Mont.—2 mg/L 4. Dext. & Mont.—2 mg/L each 5. Control—no supp. 6. Initial—sacrifice
II	27/ 7/66-11/10/66	27.2-17.2 (22.1) Decreasing Fall Temp.	21.3-24.2 (23.1)	1. Const.—2 mg/L 2. Const.—5 mg/L 3. Dext.—2 mg/L 4. Mont.—2 mg/L 5. Dext. & Mont.—2 mg/L each 6. Control—no supp. 7. Initial—sacrifice
III	21/10/66- 6/12/66	18.2- 5.6 (12.6) To Lowest Fall Temp.	22.0-23.3 (20.9)	1. Cornst.—2 mg/L 2. Cornst.—5 mg/L 3. Dext.—2 mg/L 4. Control—no supp. 5. Held in tray, York Rv. 6. Initial—sacrifice
IV	1/ 4/67-29/ 6/67	12.8-25.6 (18.2) Increasing Spring Temp.	20.7-17.9 (19.1)	1. Cornst.—0.25 mg/L 2. Cornst.—0.50 mg/L 3. Dext.—5 mg/L 4. Dext.—5 mg/L & Mont.—2 mg/L 5. Control—no supp. 6. Held in tray, York Rv. 7. Initial—sacrifice

gen levels were determined colorimetrically by the method of KEMP & KITS VAN HEIJNIGEN (1954). Analysis of Variance testing (SOKAL & ROHLF, 1969) showed that the initial mean underwater shell weights, shell heights and total volumes among the oyster groups in each experiment were statistically similar (*i. e.*, differences among the oyster groups were non-significant at the 5% significance level). Consequently it was assumed that, for each experiment, the sacrificed and experimental groups had statistically similar initial mean wet tissue weights and glycogen levels.

Oysters were held in Plexiglas troughs under running York River water. Troughs measured 36 cm long by 19 cm wide by 6 cm high and consisted of 5 compartments (4 oysters per compartment) and a baffle to insure thorough mixing of water and supplement. Oysters were oriented with bills facing the current and the right valve (flat side) up. Position of oysters in respect to the inflow was changed daily on a random basis. Troughs and oysters were cleaned of feces and pseudofeces every other day.

York River water was pumped to a large overhead trough in the laboratory, flowed into a conducting column of Plexiglas, through Tygon tubing to flow meters and then to the oyster-holding troughs through Tygon tubing. Water flow through the flow meters was checked twice a day and readjusted to the proper flow if necessary. In Experiments I, II and III, the water flow was 1 L/min.; in Experiment IV the water flow was 0.5 L/min. Water temperature and salinity were not regulated but were the same as that of the York River.

The dietary supplements were hydrolyzed cornstarch, dextrose in solution, and dextrose mixed with the clay mineral, montmorillonite. Montmorillonite alone was run as a "control" for the montmorillonite and dextrose mixtures in Experiments I and II but not in Experiment IV; the dextrose and montmorillonite mixture was not used in Experiment III. Individual supplements were prepared by mixing aliquots of either cornstarch, dextrose, montmorillonite or dextrose and montmorillonite mixed with 3 000 mL of tapwater in 4 000 mL Erlenmeyer flasks. The flasks were fitted with 2-holed rubber stoppers. One hole contained a short piece of glass tubing plugged with cotton which functioned as a vent. The second hole contained a glass tube which extended to near the bottom of the flask and served as a delivery tube. Before use, supplements in their flasks, rubber stoppers and glass tubing were autoclaved for 10 minutes at 10 pounds pressure (115° C). This treatment sterilized the supplements and suppressed bacterial growth in the holding flasks. It also converted part of the cornstarch into amylose and amylopectin through hydrolysis of the (#) 1-6 linkages.

Supplements were delivered to the experimental holding troughs through Tygon tubing attached to the glass delivery tube. Flows were regulated by peristaltic pumps and checked twice daily. Particulate supplements were kept in suspension by means of magnetic bars and stirrers. The contents of each flask lasted about 3 days and as the flasks became empty, they were replaced by full, pre-sterilized, reserve flasks.

Addition of supplements to the oyster groups was stopped 24 hours prior to the end of each experiment, and oysters received only York River water during this period so that any undigested supplement might be eliminated from their digestive tracts. Individual oysters were then cleaned of particulate debris and measured for final underwater shell weight, shell height, total volume, wet tissue weight and glycogen level by the previously described methods.

For each experiment, statistical comparisons between initial and final measurements of each oyster group were conducted to determine if significant changes in the variables of interest occurred during the course of the experiment. Comparisons between initial and final mean underwater shell weights, shell heights and total volumes were facilitated by paired "t" tests (SOKAL & ROHLF, 1969). Comparisons between mean wet tissue weights and glycogen contents of the sacrificed group to the final values of the control and test groups were carried out by single classification Analysis of Variance and the appropriate F-tests (SOKAL & ROHLF, *op. cit.*). Statistical comparisons between the final mean measurements of the control group and those of the test groups were also conducted using single classification Analysis of Variance and the appropriate F-tests. All statistical tests were evaluated at the 5% significance level.

All oyster groups were held in the laboratory with the exception of Experiments III and IV where one group per experiment was maintained in a wire tray suspended in the York River to compare "natural changes" in variables to those of the laboratory control group.

RESULTS

EXPERIMENT I

This study was conducted during mid-summer when water temperatures were maximal and after most spawning had occurred.

Oyster groups receiving the cornstarch and dextrose supplements and the dextrose and montmorillonite mixture had final glycogen levels significantly higher than

Table 2

Experiment I: initial and final measurements on oysters (7 July 66-13 Aug. 66).

Group designation	Glycogen content %	Wet tissue weight g	Shell height mm		Underwater shell weight g		Total volume cc	
			Initial	Final	Initial	Final	Initial	Final
Initial	0.51	1.42	39.6	—	4.88	—	7.01	—
Control	0.47	1.50	42.5	45.5 ¹	5.33	7.21 ¹	7.51	9.13 ¹
Cornst.—2 mg/L	1.06 ^{1 2}	1.75 ¹	40.1	45.0 ¹	5.54	8.50 ¹	7.08	9.75 ¹
Dext.—2 mg/L	1.17 ^{1 2}	1.31	38.9	44.0 ¹	5.45	7.81 ¹	7.05	9.19 ¹
Mont.—2 mg/L	0.63	1.27	37.8	41.8 ¹	5.18	7.35 ¹	7.11	8.96 ¹
Dext. & Mont.— 2 mg/L each	0.87 ^{1 2}	1.40	41.5	46.1 ¹	5.46	7.75 ¹	7.37	9.55 ¹

¹Final mean value is significantly different from the initial mean value, $\alpha = 0.05$.²Final mean value is significantly different from that of the control, $\alpha = 0.05$.

their initial mean levels (Table 2). The control group and the group receiving montmorillonite alone showed no significant change from initial levels. Only the group receiving cornstarch increased significantly in mean wet tissue weight. Final mean underwater shell weight, shell height and total volume of each oyster group were significantly greater than the initial mean values.

Comparison among final mean measurements showed that the oyster groups receiving cornstarch, dextrose and the dextrose and montmorillonite mixture had final glycogen levels significantly higher than that of the control group. Levels were respectively 2.2, 2.5, and 1.9 times greater than the control level of 0.47%. Final mean levels of the other variables of all test groups, however, were not significantly different from those of the control group.

EXPERIMENT II

This experiment was conducted during a period of falling water temperatures when oysters in nature normally begin to accumulate glycogen and spawning has ceased.

All oyster groups exhibited significant increases in all variables measured during this experiment (Table 3). Unfortunately, changes in glycogen levels could not be determined due to the failure of the freezer in which the initially sacrificed tissues were held.

Only the cornstarch and the dextrose in solution supplements produced final mean glycogen levels significantly higher than that of the control group. Final mean glycogen levels of the groups receiving cornstarch at 2 and 5 mg/L showed gains which were respectively 13.6 and 15.6

Table 3

Experiment II: initial and final measurements on oysters (27 August 1966-11 Sept. 66)

Group designation	Glycogen content %	Wet tissue weight g	Shell height mm		Underwater shell weight g		Total volume cc	
			Initial	Final	Initial	Final	Initial	Final
Initial	—	1.37	40.9	—	6.27	—	8.15	—
Control	0.68	1.88 ¹	41.5	47.8 ¹	6.08	8.40 ¹	8.31	10.45 ¹
Cornst.—2 mg/L	9.26 ²	2.92 ^{1 2}	42.0	50.1 ¹	6.15	9.99 ¹	8.19	11.96 ¹
Cornst.—5 mg/L	10.59 ²	3.24 ^{1 2}	41.2	49.2 ¹	6.28	9.70 ¹	8.56	11.99 ¹
Dext.—2 mg/L	1.62 ²	1.95 ¹	41.7	48.9 ¹	6.21	9.06 ¹	8.08	11.01 ¹
Mont.—2 mg/L	1.08	1.67 ¹	41.8	46.1 ¹	6.48	8.83 ¹	8.14	10.33 ¹
Dext. & Mont.— 2 mg/L each	1.23	2.00 ¹	42.1	47.5 ¹	6.46	9.23 ¹	8.31	11.02 ¹

¹Final mean value is significantly different from the initial mean value, $\alpha = 0.05$.²Final mean value is significantly different from that of the control, $\alpha = 0.05$.

times greater than the control level (0.68%). The group receiving dextrose in solution was only 2.4 times greater than the control level. The significantly higher glycogen levels in the cornstarch-fed groups were accompanied by significantly heavier mean wet tissue weights; this was not so for the dextrose-fed group. As in Experiment I, none of the supplements had a significant influence on shell height, underwater shell weight and total volume.

EXPERIMENT III

This study was conducted in late fall when all oyster spawning is over and oysters in nature have accumulated glycogen to maximal storage levels (based on the yearly cycle of glycogen accumulation).

Final mean measurements, with the exception of shell height, of all laboratory groups were significantly greater than initial measurements (Table 4). Only the group receiving cornstarch at 5 mg/L exhibited a significant increase in shell height. The York River group did not increase significantly in any of the variables measured.

At the end of the study, all supplements yielded final mean glycogen levels significantly higher than the control level of 2.8%. The groups receiving the cornstarch supplements had levels 3.2 (2 mg/L) and 4.1 (5 mg/L) times greater than that of the control group. The mean glycogen level of the dextrose-fed group was 1.6 times greater than the control level. However, only the cornstarch supplements resulted in final mean wet tissue weights significantly greater than that of the control group. The York River group had a final mean glycogen level and wet tissue weight which were significantly less than those of the control group, but did not differ significantly from the

control group in the other variables measured. Again, supplements had no significant influence on shell height, underwater shell weight and total volume.

EXPERIMENT IV

This study was conducted in early spring during a period of rising water temperatures. Early spring is prior to spawning and is normally a period of glycogen accumulation after winter depletion in populations of oysters occurring in nature.

All oyster groups, including the York River group, increased significantly in all variables measured during this experiment (Table 5). The cornstarch supplements produced final mean glycogen levels significantly higher than that of the laboratory control group. These levels were approximately 1.5 times greater than the control level of 3.08%. Dextrose in both supplemental forms had no significant influence on glycogen levels. None of the supplements had a significant influence on mean wet tissue weights, shell heights, underwater shell weights and total volume. The oyster group held in the York River had final mean glycogen level significantly less than that of the control group, but did not significantly differ from the control group in the other variables measured.

DISCUSSION

It is assumed in this study, that significant differences between test and control oysters in final measurements were primarily due to the addition of supplements and not to adverse laboratory conditions. This assumption is sup-

Table 4

Experiment III: initial and final measurements on oysters (21 Oct. 66-6 Dec. 66).

Group designation	Glycogen content %	Wet tissue weight g	Shell height mm		Underwater shell weight g		Total volume cc	
			Initial	Final	Initial	Final	Initial	Final
Initial	1.84	1.86	43.0	—	6.73	—	9.00	—
York River	2.08 ²	1.80 ²	45.5	45.0	6.57	7.28	9.48	9.83
Control	2.84 ¹	2.31 ¹	44.1	46.4	6.92	8.43 ¹	9.48	10.55 ¹
Cornst.—2 mg/L	9.13 ^{1 2}	3.10 ^{1 2}	44.2	46.6	6.92	8.43 ¹	9.55	10.79 ¹
Cornst.—5 mg/L	11.48 ^{1 2}	3.09 ^{1 2}	44.9	47.3 ¹	6.87	8.32 ¹	9.84	10.90 ¹
Dext.—2 mg/L	4.64 ^{1 2}	2.49 ¹	44.5	46.3	6.81	8.13 ¹	9.29	10.43 ¹

¹Final mean value is significantly different from the initial mean value, $\alpha = 0.05$.

²Final mean value is significantly different from that of the control, $\alpha = 0.05$.

Table 5

Experiment IV: initial and final measurements on oysters (1 April 67-19 June 67).

Group designation	Glycogen content %	Wet tissue weight g	Shell height mm		Underwater shell weight g		Total volume cc	
			Initial	Final	Initial	Final	Initial	Final
Initial	1.67	1.91	46.1	—	6.88	—	9.84	—
York River	2.33 ¹ ²	2.94 ¹	44.4	47.8 ¹	6.82	10.30 ¹	9.76	12.24 ¹
Control	3.08 ¹	2.46 ¹	44.2	49.3 ¹	6.98	9.47 ¹	9.44	11.32 ¹
Cornst.—0.25 mg/L	4.58 ¹ ²	2.33 ¹	43.8	50.6 ¹	6.98	9.59 ¹	9.30	11.60 ¹
Cornst.—0.50 mg/L	4.73 ¹ ²	2.83 ¹	45.8	52.3 ¹	6.68	9.47 ¹	9.86	12.33 ¹
Dext.—5 mg/L	3.48 ¹	2.69 ¹	45.2	50.9 ¹	6.96	9.91 ¹	9.52	12.09 ¹
Dext.—5 mg/L & Mont.—2 mg/L	3.02 ¹	2.49 ¹	44.8	50.8 ¹	7.08	10.10 ¹	9.45	12.02 ¹

¹Final mean value is significantly different from the initial mean value, $\alpha = 0.05$.²Final mean value is significantly different from that of the control, $\alpha = 0.05$.

ported by Experiments III and IV in which control oysters held in the laboratory showed increases equal to or exceeding those of oysters held in the York River.

Our studies showed that effects of carbohydrate supplements on glycogen levels varied with season. Seasonal changes in glycogen content are typical of oysters occurring naturally in Chesapeake Bay (GALTSOFF, CHIPMAN, ENGLE & CALDERWOOD, 1947; ENGLE, 1950). These changes were reflected in glycogen levels among the control groups held in the laboratory; percentage change and absolute levels were lower in summer and early fall than they were in late fall and early spring. This agrees with known aspects with the oyster's spawning cycle. Fall and spring are periods of glycogen storage, while stored glycogen is utilized in the formation of sexual products during the summer months. Effects of cornstarch and dextrose in the fall and spring on glycogen levels paralleled this natural cycle. The oysters receiving cornstarch during these periods of glycogen storage had substantially higher glycogen levels than those normally found in oysters occurring in the natural environment. Consequently, utilization of carbohydrates as feeding supplements must be considered in relation to the natural glycogen cycle.

Comparison of the cornstarch and dextrose results clearly shows that cornstarch was the better supplemental food. Its effect was most noticeable in the fall. At this time concentrations of cornstarch ranging from 2 to 5 mg/L greatly influenced both glycogen levels and wet tissue weights, but dextrose influenced only glycogen levels and to a much lesser extent. HAVEN (1965) and GILLESPIE *et al.* (1966) concluded from their results that dextrose was limited in value as a carbohydrate supplement for oysters. It is noted that cornstarch at 2 and 5 mg/L in both fall experiments resulted in similar levels of percentage glyco-

gen (approximately 9 to 11%). This suggests that cornstarch concentrations much over 2 mg/L could not be assimilated into stored glycogen.

The effect of mixture of dextrose and montmorillonite was, in absolute terms, minimal, the only positive influence on glycogen levels occurring in the summer experiment. Assuming that the dextrose was adsorbed on the clay, it is postulated that the oysters were not capable of stripping the adsorbed dextrose off the clay particles. Another possibility is that the uptake route of glucose is through bacteria and that bacteria can not strip the dextrose off the clay particles.

Cornstarch at the low concentrations of 0.25 and 0.50 mg/L in the spring experiments influenced glycogen levels, yields being only about one-half of those of the 2 and 5 mg/L concentrations in the fall experiments. This point is emphasized since it shows that during spring, cornstarch concentrations of only one-tenth of those used during the preceding fall yielded one-half of the glycogen yielded by the higher concentrations. It is also emphasized that during the spring study, water flows were only one-half of those used in the preceding study.

The lack of influence of cornstarch on shell height, underwater shell weight and total volume at any season agrees with the data of HAVEN (1965). GILLESPIE *et al.* (1966) concluded that cornstarch influenced various parameters of shell size as well as glycogen levels and tissue weight and volume. However, the lack of appropriate statistical comparisons in the presentation of their data makes their contention difficult to support. It is concluded that the influence of cornstarch as a supplement for oysters is limited to glycogen content and tissue size with the major influence being on glycogen levels. Thus, supplements which influence glycogen levels to a high degree

can be expected to have a similar influence on tissue size. This relation supports the statement of MITCHELL (1917) that investigations leading to increased meat yields must consider supplements which influence glycogen formation.

HAVEN (1965) pointed out that supplemental feeding with cornstarch offered promise as a cultural technique for increasing meat production. Recently the method has been used along with an algal supplement on a routine basis to condition oysters prior to their spawning in a hatchery (DUPUY & RIVKIN, 1972).

It is quite probable that the seasonal changes in meat quality of oysters observed by HAVEN (1962) are associated with the natural fluctuation of a particulate carbohydrate in estuarine waters. Possible sources of this substance might be algal cells or detrital material originating from the breakdown of grasses such as *Spartina* or *Zostera*.

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