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Investigations with monozygous twin dairy heifers

Roy Orlando Thomas

To the Graduate Council:

I am submitting herewith a thesis written by Roy Orlando Thomas entitled "Investigations with monozygous twin dairy heifers." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Eric W. Swanson, Major Professor

We have read this thesis and recommend its acceptance:

R. H. Lush, S. A. Hinton

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

March 1, 1952

To the Graduate Council:

I am submitting to you a thesis written by Roy Orlando Thomas entitled "Investigations with Monozygous Twin Dairy Heifers." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science with a major in Dairying.

Eric W. Swanson
Major Professor

We have read this thesis
and recommend its acceptance:

R. H. Lush

S. A. Hinton

Accepted for the Council:

E. G. Waters
Dean of the Graduate School

INVESTIGATIONS WITH MONOZYGOUS TWIN DAIRY HEIFERS

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

Roy Orlando Thomas

March 1952

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INTRODUCTION

Monozygous twins have been reported to have a higher value than non-twins for experimental studies comparing two treatments, because these twins have no inherited differences. Differences which develop between identical twins are due to environment. Physiological processes such as growth and milk production are very complex and are regulated in some degree by more simple processes. Since monozygous twins are expected to react similarly in growth and production, they should also be expected to react similarly in less complex functions. Two physiological processes of particular interest in dairy cattle are the efficiency of feed utilization and the adaptability to high ambient temperatures. This investigation was planned to measure the differences in the physiological processes of monozygotic dairy heifers under the same environment and compare these differences with ones found within pairs of selected animals of the same age and weight. Growth of identical twin pairs fed at the same level and other pairs of identical twins at different levels of nutrition was measured. All data were compared to determine the value of identical twins for experimental observations involving physiological processes.

LITERATURE

Experimental Use of Identical Twin Cattle

Until about 1920 identical twinning in cattle was believed to occur rarely if ever. Lillie (43, 44), reporting on the free-martin, probably started the interest in monozygotic twins for research purposes. According to Bonnier and Hansson (6), in 1930 Kronacher and co-workers in Germany made the first collection of identical twin cattle for experimental purposes. This work was never extended further than the study of the recognition of monozygosity within the twin pair. Swedish workers in 1938 started collecting monozygous pairs of heifers for planned experimental studies. Hancock (29) reported that there were over 200 sets collected in New Zealand for experimental purposes. In the United States, Chandrakant et al. (12), Olson and Petersen (47) and Petersen et al. (48) have reported upon the use of identical twins and triplets in experiments.

Hayden (34) reported a study of one blood line of cows in which twins were dropped regularly. Lush (45) reported a case of twinning which indicated that the sire had a greater influence on twinning than did the dam. These results indicate that twinning in cattle is hereditary. Animal Research (1) reported that identical twinning was not determined by heredity as was fraternal twinning. Hancock (29) reported that one pair of twins occurred in 100 births in New Zealand cattle. Animal Research (1) reported the rate of twinning was 3.5 percent for Friesian, 1.8 percent for Ayrshire, 1.0 percent for Jersey and 0.5 percent for beef breeds in New Zealand. This station had located two sets of identical triplets in six years of collecting identical animals. It was calculated that one set of identical triplets occurred in 4,000,000 births. Bonnier and Skarman (10) reported that 10 percent of like sexed twins in cattle were identical. Bonnier (4, 5) calculated that one set of identical female twins occurred in 2,000 births in Swedish cattle.

Identical twins have been used to determine the environmental influence on the production of fat and milk. Hancock (29) reported an average difference of eight pounds in fat production within nine pairs of identical twin cows for a complete season. An anonymous report of New Zealand experiments (2) listed the butter fat production of nine sets of identical twins. The greatest difference of production within pairs was 14 pounds, while the range between pairs was from 6 and 11 to 266 and 280 pounds. Hansson (30) reported that production from one twin with the udder partially destroyed was not as high as that of her sister, although the parts of the udder remaining increased production as compared to the corresponding parts of the other twin. Hansson (32) reported that premature calving (9 days) of one of a set of identical twin heifers depressed milk production. Fat and other organic matter content of the milk was increased, but this increase did not bring total fat or organic matter production to the level of the mate. Hansson and Bonnier (33) found that milk production of one twin was increased by 6.4 percent due to three milkings per day as compared to the other twin milked two times per day. The greatest difference was found between pairs under the same conditions. Petersen _et al._ (48) reported on the use of identical triplets in studying the yield of milk and butterfat and the utilization of feed when animals were milked one, two and three times per day. Hansson (31) found that feeding excessive amounts of calcium and phosphorus decreased total production of milk by high-producing cows while medium and low-producing cows' production of milk was little affected. The production of these cows was compared to their identical twins under normal feeding levels of calcium and phosphorus.

Bonnier and Hansson (8) found that milk of identical twins was statistically equal in protein at fixed fat levels. Protein was not equal for fraternal twins, and very unequal for non-related animals at fixed fat levels. Jarl (36) reported that the variation in ascorbic acid content of milk was 2.14 times greater for dizygotic twins than for identical twins. Winzenried and Wanntorp (65), using identical twins, decided that vitamin A, carotene, and carotenoid content of milk was genetically determined, and that the upper limit of conversion of carotene into vitamin A was determined by heredity.

Bonnier and Hansson (6) and Bonnier, Hansson and Skjervold (9) found that high feeding during growth and the first gestation increased the yield of milk over low feeding. Flux (17) reported that low feeding of two-year-old heifers in the latter part of the first gestation period decreased the yield of milk by an average of 1,057 pounds as compared to the yield of identical heifers under a normal level of feeding.

Olson and Petersen (47) reported that identical triplet bulls reacted alike physically, and that no significant difference was found in the semen except for motility and numbers of abnormal sperm for a 16-week period at the start of the experiment.

Hancock (28) reported that pairs of identical twin cows had practically the same grazing habits. Grazing and ruminating time, distance walked, refusals of forage, speed of ruminating and grazing, number of bites per bolus, and number of micturitions and defecations were characteristic for a pair of twins.

In a trial to determine the effects of the quality of hay upon growth, Chandrakant et al. (12) found that identical twins fed different quality hays grew at rates as near the same as did non-twins fed the same hay. Bonnier and Hansson (7) and Bonnier, Hansson and Skjervold (9) found that the growth within pairs of identical twins on different levels of feeding was nearer the same than between pairs on the same level of feeding. The planned feeding levels were 87.5 and 112.5 percent and 67 and 133 percent of normal. In the 67 and 133 percent level Bonnier, Hansson and Skjervold (9) found that cessation of growth was genetically determined, although low feeding would delay somewhat the age at which growth ceased. In these reports 18 pairs of twins fed at different levels were compared, and seven pairs were used as controls. Measurements in these growth comparisons showed that the head would develop more fully at lower levels of nutrition than would other parts of the body. Full development was progressively less toward the rear. Individuals of a pair on different planes of nutrition showed no significant difference between measurements when comparisons at the same weight were made.

Bonnier and Hansson (6, 7) and Hansson (30) reported almost equal growth for identical twins under the same environmental feeding conditions.

Hansson (30) reported that there was only one kilogram difference in the carcass weights of identical twins slaughtered in their second lactations. These animals were fed according to milk production after first calving. The carcass weights showed that the high fed animal was heavier due to more fill from the extra feed.

Hancock (29) reported that one set of identical twins was equal to 20 pairs of ordinary animals for investigations of factors affecting growth, and 40 or 50 pairs for investigations of factors affecting the production of fat. Another New Zealand report (2) concluded that one set of identical twins was equal to 50 ordinary calves for growth comparisons, and one set was equal to 100 cows for production comparisons. Flux (17) calculated that one pair of identical twins was equal to 28 pairs of unrelated animals for comparisons of the fat content of milk. Bonnier and Hansson (6) reported that one set of identical twins was equal to 20 pairs of calves for growth and 50 pairs of cows for production studies.

Digestibility of Forages

Crampton (14) stated that digestibility of pastured forage was hard to determine because the intake of grazing animals was qualitatively and quantitatively difficult to measure. Quantitative feces collection also presented a troublesome problem. An indicator (chromium or other material) would be of value in studying the digestibility of pastured forage, for such indicator substance would eliminate the necessity of cumbersome feces collection apparatus. Also, the animal would be under natural conditions. Chromic oxide, protein, lignin, silica, and chromogen(s) have been reported as indicators by Schürch, Lloyd and Crampton (58), Forbes (19), Forbes and Garrigus (20), Gallup, Hobbs and Briggs (25) and Reid et al. (52, 53) to give results comparable to the total collection method. Schürch, Lloyd and Crampton (58) used rats to find that average results with chromic oxide as an indicator were comparable to the total collection technique for determining digestibility. Forbes (19) found that protein as an indicator gave results within limits of the lignin-ratio or total collection techniques. On the other hand, Forbes and Garrigus (20) thought that protein as an indicator was probably due more to the high negative relationship between lignin and protein contents than to any effects of protein upon digestibility. Forbes (19) reported that unaccountable variations occurred in the lignin-ratio technique of determining the digestibility of forages. Ellis, Matrone and Maynard (16) found that lignin gave results comparable to the conventional method if lignin were determined by the 72 percent H_2SO_4 method. Gallup, Hobbs and Briggs (25) found that silica gave good results in determining digestibility of forage by animals confined. Feces collected in sacks from animals in lots or from animals from which the feces was not protected contained more silica than was in the forage consumed. Feces collected from animals on pasture had 36 percent more silica than the calculated forage intake contained. Reid et al. (52) found that naturally occurring chromogen(s) which absorbed light at 406 mμ gave results comparable to the total collection method of determining digestibility of forages. The recovery in the feces of the chromogenic material consumed ranged from 94.2 to 106.2 percent in nine digestion trials.

The averages for the trials ranged from 99.5 to 102.7 percent of the intake. Hays and fresh pasture forages were used. Reid et al. (51) extended the chromogen technique to make possible the use of this method for determining the digestibility of pastured forages. Kane et al. (38) reported that lignin was not reliable as an indicator for orchardgrass digestion studies. On the other hand, chromium oxide and plant pigments gave good results compared to the conventional method. Cook and Harris (13) found the chromogen technique to be unreliable for determining the digestibility of some desert forages. Chromogen and lignin gave results very close together for alfalfa hay in the same series of experiments.

McCullough et al. (46) reported that dry matter digestion coefficients ranged from 52 to 94 percent, with an average of 75 percent for the season, from pasture plots checking the effects of manure on digestibility of the plants. The protein coefficients ranged from 59 to 88 percent with an average of 70.2 percent. The season extended from May to October. Crampton and Jackson (15) reported an average of 71 percent digestibility of the dry matter of pasture herbage by steers. The range in dry matter digestibility was from 68 to 80 percent. Protein digestibility averaged 76 percent with a range of 72 to 79 percent. Hodgson and Knott (35) found the dry matter and protein of artificially dried pasture forages to be digested by dairy heifers from 65.35 to 69.11 percent and 74.08 to 76.42 percent respectively. Protein content of the forage on a dry matter basis was 24.64 percent. The heifer having the low coefficient of digestion was in better condition than the other two heifers. Groenewald et al. (26) found no consistence between five animals in digestion of dry matter for different digestion trials using lucerne hay. Digestion coefficients ranged from 56.3 to 59.3 percent. Protein digestion coefficients varied little between trials or animals. Range in protein digestibility was 75.0 to 75.5 percent. Forbes et al. (18) found that the range in digestibility of clover-timothy hay by sheep was 55.1 to 58.9 percent of the dry matter and 55.7 to 61.4 percent of the crude protein. Ellis, Matrone and Maynard (16) reported 62.7 ± 0.34 and 62.0 ± 0.40 per cent as the digestibility of the dry matter of sudan-grass by sheep. Crude protein coefficients were 64.8 ± 0.40 and 62.8 ± 0.73 percent. Forbes and Garrigus (20) gave the range in digestibility of dry matter of Ky. 31 fescue, orchardgrass, ladino, and alfalfa to be 62 to 85 percent, 64 to 81 percent, 83 to 86 percent and 71 to 79 percent respectively. The protein digestibility of the forages in the same order were 59 to 80 percent, 68 to 78 percent, 82 to 84 percent and 75 to 80 percent. These plants were pastured by Hereford steers weighing 625 to 650 pounds. A Joint Committee (37) gave the average digestion coefficient of mixed pastures from 45 reviewed digestion experiments as 72 percent for dry matter and 75 percent for crude protein.

Gallup and Hobbs (24) found a 10 percent error due to weighing uncovered dishes when determining dry matter content of feces. Four to 11 percent errors were found in the nitrogen determinations of dried feces even though acid alcohol was used to prevent loss of nitrogen. Accurate ash determinations were obtained from fresh samples if temperature of ashing was between 600 and 650° F.

Hamilton et al. (27) found that only three or four days were required for a constant level of a feed indicator (chromic or ferric oxide) to be attained in the feces of animals.

Heat Tolerance

Brody (11), Fuller (21), Seath (59) and Gaalaas (23) reported that breeds of cattle differ in their abilities to adjust to high environmental temperatures, and that individual animals within breeds vary in their abilities to adjust to unfavorable atmospheric conditions. Rhoad (54) reported that the distribution of breeds in the United States was due in part to the differences of the breeds in heat tolerance. Measures that have been used to determine the heat tolerance of animals are body temperature, respiration rate, pulse rate and production.

Respiration Rate

Fuller (21) summarized the factors affecting the respiration rate of cattle as follows:

Age--Young have highest rate
Exercise--Accelerates
Condition of nervous system--Excitement accelerates
Temperature--Warmer temperature accelerates
Ingestion of food--Rate higher when abdomen is very full
Period of day--Generally lowest in morning, highest in evening

Regan and Richardson (50) reported that the respiration rate of cattle followed Van't Hoff's law of chemical reaction, that is, 10^{o} C. (18^{o} F.) rise in temperature doubled the rate. Kibler, Brody and Worstell (42) reported that the respiration rate of Jersey cows was 5 to 6 times as high at 100° F. as at 50° F. room temperature. Kibler and Brody (40) found that the respiration rate of cows varied with air temperature. The rates were 10 to 15 per minute at 5° F., 20 to 30 per minute at 50° F., and over 100 per minute at 95° F.

Kibler and Brody (41) reported that respiration rate rose sharply between 69° and 90° F. environmental temperature. Above 90° F. the rates tended to reach a maximum for European breeds (Jersey and Holstein). Above 80° F. the rate rose rapidly for Brahman, but showed no tendency to reach a maximum up to 105° F. room temperature. The respiration rate was higher for Brahman at 105° F ambient temperature than for Jersey and Holstein. Kelley and Rupel (39) reported that at low temperatures the respiration rate changes with environmental temperature. Gaalaas (22) found a significant correlation between respiration rate of Jersey cows and ambient temperature in the temperature range of 33° to 95° F. An increase in respiration was first noticed at 51° F., with a more rapid increase above 69° F. air temperature. Respiration rates ranged from 8 to 78 per minute below 69° F. and 16 to 127 per minute above 69° F. Riek and Lee (55, 56) reported a rise in respiration rate of Jersey cows in milk from 25.4 ± 6.1 at normal temperatures to as high as 160 per minute at high temperatures and humidities (95 to 110° F. and 12 to 16 grams per cubic foot). On the other hand, young grade Jersey calves showed a rise from 21.2 ± 4.4 at normal temperatures to a maximum of 256 per minute at high temperatures. The respiration rate increased more rapidly for the calves than for the cows. Respiration rates tended to level off at temperatures below 95° F. but kept rising due to exposure to temperatures above 95° F.

Seath and Miller (62) found that cows and heifers increased their respiration rates due to exposure to direct sunlight for two hours. The rate of the heifers was less affected than that of the cows. Gaalaas (22) reported an increase of 20 respirations per minute for Jersey cows exposed to the sun without shade.

For cows exposed to the sun for two hours, Seath and Miller (60) found that shade reduced the respiration rate by 27.2 per minute in 30 minutes and by 25.2 per minute in one hour. Shade with sprinkling reduced the respirations by 49.4 per minute in 30 minutes and by 41.2 per minute in one hour. This is 81 and 61 per cent greater than shade alone. Seath and Miller (62) found that shade and shade with sprinkling caused a smaller decrease in the respiration rate of heifers than of cows.

Seath and Miller (61) suggested that six respiration observations under atmospheric conditions were sufficient to select animals for heat tolerance.

Fuller (21) reported that the average respiration rate of cows was 21.7 per minute, while high producing cows averaged 28.9 per minute. Gaalaas (22) found that 82 per cent of the Jersey cows studied had respiration rates between 16 and 28 per minute at 50 to 60^{o} F. air temperature. Riek and Lee (55, 56) reported that 25.4 $\pm$ 6.1 and 21.2 $\pm$ 4.4 were normal respiration rates for Jersey cows and young grade Jersey calves respectively.

Robinson and Lee (57) found a significant correlation between high feed intake and respiration rate. Seath and Miller (61) reported a rise in respiration rate of cows walked to a barn.

Body Temperature

Kibler and Brody (40) reported that rectal temperatures of cows are constant between 5 and 70° F. air temperatures. Above 70° F. air temperature the rectal temperatures increased. Kibler, Brody and Worstell (42) found that rectal temperatures started to rise between 70 and 80° F. environmental temperatures. Regan and Richardson (50) found that body temperatures of cows remained constant below environmental temperatures of 70° F., but above 70° F. body temperatures increased. Gaalaas (22) found that body temperatures of cows remained constant below 70° F. environmental temperature. Consistent increases in body temperature were found for increases in environmental temperature above 70° F.

Kibler, Brody and Worstell (42) found that rectal temperatures reached 108° F. for Holstein cows and 106° F. for Jersey cows at 105° F. room temperature. Kibler and Brody (41) reported that at 105° F. room temperature the rectal temperature of Jersey and Holstein cows was above 108° F., while that of Brahman cows was above 106° F.

Riek and Lee (55) found that an equilibrium in rectal temperature of cows was reached when air temperatures were between 85 and 95° F. Between 95 and 110° F. an equilibrium was reached more slowly. Seath and Miller (62) found that the body temperatures of heifers did not vary as much as did the body temperatures of cows under the same environmental conditions. On the other hand, Riek and Lee (56) found that young grade Jersey calves were more affected by high temperatures than were cows. Seath and Miller (62) found that the body temperature of cows and heifers was increased due to exposure of the animals to direct sunlight for two hours. Gaalaas (22) found an increase of 0.7° F. in body temperature of cows exposed to the sun for extended periods.

Seath and Miller (62) reported that rectal temperature of cows after exposure to the sun decreased from 104.08 to 101.91° F. due to shade and from 104.08 to 100.76° F. due to shade and sprinkling. Heifers under the same conditions showed a decrease in rectal temperatures from 102.99 to 102.18° F. due to shade, and from 102.99 to 101.82° F. due to shade and sprinkling. Seath and Miller (60) reported that shade alone was effective in materially reducing rectal temperature of cows exposed to the sun for two hours. Shade and sprinkling caused a quicker return of rectal temperatures to normal values. Sinha and Minett (63) reported decreases of 0.68 and 0.73° F. in body temperatures of water buffalo due to splashing with water.

Seath and Miller (61) suggested that seven body temperature observations under atmospheric conditions were sufficient to determine the heat tolerance of animals.

Riek and Lee (55, 56) reported that the average normal temperature of young grade Jersey calves was 101.61 ± 0.69° F., and that of Jersey cows in milk was 101.17 ± 0.67° F. Gaalaas (22) reported that the average normal rectal temperature of Jersey cows was 101.06 ± 0.46° F.

EXPERIMENTAL PROCEDURE

Swedish (6, 9) and New Zealand (1, 2, 29) investigators have reported that identical twin cattle grow and produce milk at very similar rates under the same or similar environment. In order to do this their physiological processes would have to be very much alike, if not identical. In this experiment observations were made of the similarity of identical twins and non-twin pairs in the efficiency of forage utilization (digestion) and the response to high ambient temperatures. In addition to these experiments, the effect of unlike rations on growth rates of other sets of identical twins are reported. A limited study with the latter group was made on the effect of the feeding level upon heat tolerance.

Animals

The animals used in these observations were six pairs of identical twin dairy heifers and two pairs of selected individuals. The identical twins were obtained from farmers in Tennessee. The selected animals were picked from the Jersey herd owned by the University of Tennessee.

Three pairs of identical twins, two pairs of grade Jerseys and one pair of purebred Jerseys, were used in the digestion and heat tolerance observations. Data relative to growth of these twins was also recorded. Two pairs of non-twin heifers were selected to compare in age and weight with the identical twins in order to measure the uniformity of response of twins and non-twins.

Three other pairs of identical twins, two pairs of grade Jerseys and one pair of grade Holsteins were divided into two groups, one from each pair in each group. These groups were fed unlike rations. One ration was similar to the rations fed the animals in the University of Tennessee herd to produce normal growth. Hay was fed unlimited, but grain was limited. The other group was fed a limited amount of hay. A special pelleted, high energy grain mixture was fed this group free choice.

Tables I and II give age, number and other data on the animals used in this study.

Digestibility of Forages

Rations

Four all-roughage rations were fed. Ration 1 consisted of Ladino clover, orchardgrass, and fescue pasture. This field was divided into Ladino clover-orchardgrass and Ladino clover-fescue sections. Ration 2 consisted of sudangrass plus a considerable amount of wild grasses. The sudan grass was second growth forage. Grazing by milking cows had been discontinued approximately one week before the start of grazing for this trial. Alfalfa-orchardgrass hay for ration 3 was selected and chopped with a hammer mill. Sufficient hay was chopped and stored in bags to feed the animals throughout the trial period. Ration 4 was young, tender second growth alfalfa pasture that contained some volunteer grasses.

TABLE I

DATA ON ANIMALS OBSERVED IN DIGESTION OF FORAGE AND HEAT TOLERANCE

Number			Weight		
Tag	Tattoo	Birth Date	7/10/51	10/2/51	Breeding
1	B42	Feb. 10, 1950	473	524	Purebred Jersey
2	B43	Feb. 10, 1950	489	568	Purebred Jersey
20	100	Nov. 27, 1950	265	347	Grade Jersey
21	101	Nov. 27, 1950	259	342	Grade Jersey
22	110	Nov. 20, 1950	263	356	Grade Jersey
23	111	Nov. 20, 1950	262	360	Grade Jersey
6	T296	Oct. 18, 1949	600	620	Purebred Jersey
9	T297	Nov. 27, 1949	560	630	Purebred Jersey
24	T312	Sept. 12, 1950	290	310	Purebred Jersey
25	T316	Nov. 25, 1950	320	365	Purebred Jersey
26*	T318	Dec. 4, 1950		250	Purebred Jersey

*In ration 4, non-twin 26 was substituted for non-twin 24 due to factors not under the control of the investigator.

TABLE II

DATA ON ANIMALS OBSERVED IN GROWTH AND HEAT TOLERANCE
(While Receiving Unlike Rations)

Number Tag and Tattoo	Feeding Level	Birth Date	Weight 5/29/51	Weight 11/6/51	Breeding
10	High	Jan. 24, 1951	141	385	Grade Jersey
11	Low	Jan. 24, 1951	146	344	Grade Jersey
12	Low	Jan. 24, 1951	162	409	Grade Holstein
13	High	Jan. 24, 1951	159	448	Grade Holstein
14	Low	Feb. 5, 1951	147	321	Grade Jersey
15	High	Feb. 5, 1951	164	390	Grade Jersey

Scheduling of Feeding

Table III gives the schedule for the feeding of the four rations. The heifers were fed mixed hay and grain in the periods between digestion trials, which were usually one week. At the end of each feeding period a feces sample was collected manually from each animal and put in a tightly closed glass jar.

Preparation of Samples

Samples were stored in a household type refrigerator as soon as possible after collection. The samples were kept until all analyses were finished. The forage samples for pasture trials were cut the day before feces collection. An attempt was made to collect samples represantative of the forage being consumed by the animals. Grazing animals were observed several times during the feeding period to guide the sampling of the forage. Samples of the grass were chopped with a food chopper or cut into small pieces with scissors and samples for chromogen and dry matter content were taken immediately on the fresh material. The remainder was air dried and ground with a Wiley mill to pass a 40-mesh screen for further analyses. The hay forage was sampled by taking a portion from each bag of chopped hay. This composite was rechopped with the hammer mill, mixed and a large sample taken. This sample was ground in the Wiley mill. All air dried samples were stored in closed containers.

Chromogen Analysis

Chromogen analysis was made in accordance with the method of Reid et al. (52). Five gram samples were used of the feces and green forage.

TABLE III

SCHEDULE OF FEEDING
(Digestion Trials)

Date	Ration
July 10-17	Ration 1--Ladino clover, orchard-grass, fescue pasture.
July 17-24	Intermediate period--grain and roughage.
July 24-31	Ration 2--sudangrass pasture.
July 31-Aug. 3	Intermediate period--grain and roughage.
Aug. 3-7	Intermediate period--grain and chopped alfalfa-orchardgrass hay.
Aug. 7-14	Ration 3--chopped alfalfa-orchardgrass hay.
Aug. 14-21	Intermediate period--grain and roughage.
Aug. 21-28	Ration 4--alfalfa, grass pasture.

A two-gram sample was used of the hay. Feces samples were extracted in two periods of 8 to 10 minutes duration with approximately 200 milliliters of 85 percent acetone. The extract was filtered through a Buchner funnel under slight vacuum. Grass and hay were extracted 3 and 4 periods respectively of 8 to 10 minutes. The hay sample was soaked several hours in a small amount of water before extraction. Equipment used was rinsed with 85 percent acetone which was added to the original extract. The feces extracts were made to 500 milliliter volume, and hay and grass extracts were made to 1,000 milliliter volume. After bringing to volume, all extractions were well mixed by shaking and inverting the flasks. All chromogen extractions were made within 12 hours of collection of samples, except the hay sample which was stored until the day before feces collection for extraction. Extractions were stored in a dark cabinet until the equipment was available to make readings. Hay and feces extractions from trial 3 were stored six days, while all other extractions were read within three days.

Other Analyses

Dry matter, ash and protein analyses were made in duplicate according to Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (3). Five-gram samples were used for all wet samples. Two-gram samples were used for air dried forage analysis.

Calculation of Digestibility

Calculations of the apparent digestibility of dry matter and crude protein were made by the equation, $100 - \left\{100 \frac{a \cdot x \text{ in feces}}{b \cdot x \text{ in forage}}\right\} =$ percent apparent digestibility where a equals the units of chromogen in forage, b equals the units of chromogen in feces and x equals the percent of specific nutrient.

Calculations of apparent digestibility were made for dry matter using the chromogen content arrived at by the equation presented by Reid et al. (51). This equation allows the animal to do the sampling of the forage. The formula: $y = (0.0925\ x) + (137.3383 \cdot \log x) - 242.1181$ apparent digestibility $= 100 - (\frac{y}{x} \cdot 100)$, where y equals chromogen content of forage and x equals chromogen content of the fecal dry matter. Since the self-sampling formula was determined on the basis of different chromogen units than those arbitrarily adopted in the University of Tennessee laboratory, correction was made of all units to make them equivalent to Reid's units.

Heat Tolerance

Days when the expected temperature was 90° F. or above were preferred for heat tolerance observations. Respiration rates and rectal temperatures were determined at the end of each of three one-hour periods. During the first period the animals were in the shade of a shed. This period was to allow the animals to return to normal after the excitement of handling. The animals were tied to a woven wire fence in the direct sunlight during the second period where they were required to remain standing. The animals were returned to the shed for the third period where they were allowed to lie down. The animals that were lying down were required to stand five to ten minutes before observations were made.

The paired animals were kept together during the observations and pairs were alternated to prevent one pair from remaining in the sun or in the shade a longer total time than another pair during the ten observations. Because all animals could not be tied so as to have access to water, none were allowed water during the time required for an observation.

Respirations were counted by flank movements in 15, 30 or 60 second intervals. Total time that respirations were counted was 60 seconds. Doubtful counts were repeated to assure an accurate measurement.

Rectal temperatures were obtained with a clinical thermometer inserted full length (3 inches) into the rectum. The respiration count was made on an animal before the temperature was taken.

Ambient temperatures were determined for each period by placing a thermometer near the animals under the same conditions.

Growth

Body weight was determined at weekly intervals with scales accurate to one pound. Weighing was done at the same time of the day in as far as possible. Weights were taken on three consecutive days at the beginning and at the end of the experiment. An average of these weights was considered as true weight.

Measurements of heart girth and withers height were made biweekly at the same time weighing was done. Two or three height at withers measurements were taken and averaged for each measurement recorded. Measurements were recorded to the nearest one-tenth inch.

Statistical Analyses

Statistical analyses of the differences of the apparent digestion coefficients of crude protein and dry matter were made to determine the significance of the data.

Means and standard deviations of the increase in respiration rates due to exposure of the animals to direct sunlight and the decrease in respiration rates due to shade were calculated.

RESULTS

Digestibility of Forages

The apparent digestion coefficients for dry matter calculated by the two methods previously described are given in Table IV. The automatic sampling formula developed by Reid et al. (51) grouped the digestibility figures closer than the standard formula. In ration 1 the range in digestibility of dry matter was 65.54 percent to 70.84 percent for the automatic sampling calculations, whereas, the standard equation gave a range of from 51.30 percent to 67.56 percent. For ration 1 the automatic sampling formula increased the average for the ten animals, but decreased the average for the other two pastured rations 2 and 4. The automatic sampling calculations gave figures too high for the hay forage ration 3. For this reason, in the statistical analyses, the results from the standard equation were used for ration 3. The difference in the digestibility of dry matter found within identical twin pairs was not statistically significant, although a highly significant difference ($P < 0.01$) was found between twin pairs. On the other hand no significant difference was found within or between pairs of selected non-twin animals in the digestibility of dry matter.

Table V gives the results in apparent digestibility of protein by the dairy heifers. The apparent digestibility figure for twin 20 for ration 1 is believed incorrect due to a mistake in nitrogen analysis of the feces of this heifer. A calculated figure was used in statistical evaluation of these results. A highly significant difference was found

TABLE IV

APPARENT DIGESTIBILITY OF DRY MATTER

Animal Number		Ladino, Grass Pasture	Sudan-grass Pasture	Alfalfa, Orchard-grass Hay	Alfalfa, Grass Pasture
		Automatic sampling			
Twin	1	70.84	74.86	77.44	71.29
	2	69.96	75.26	77.27	71.43
Twin	20	69.65	72.18	76.06	68.07
	21	70.45	73.23	76.73	69.19
Twin	22	65.55	72.10	76.96	69.77
	23	65.54	73.19	75.84	70.21
Non-Twin	6	68.22	71.36	76.18	69.46
	9	67.83	72.50	77.44	67.84
Non-Twin	24	66.67	74.85	75.88	
	25	66.27	74.94	76.40	69.47
	26				69.74
Average		68.10	73.45	76.62	69.65
		Standard Equation			
Twin	1	67.56	78.77	52.00	80.51
	2	65.16	79.56	51.08	80.73
Twin	20	64.85	72.98	44.50	74.85
	21	66.50	75.33	48.20	76.94
Twin	22	51.33	72.80	49.46	77.96
	23	51.30	75.25	43.24	78.71
Non-Twin	6	59.27	71.04	45.18	77.42
	9	58.90	73.70	51.96	74.41
Non-Twin	24	55.14	78.74	43.52	
	25	53.83	78.93	46.42	77.44
	26				77.91
Average		59.38	75.71	47.56	77.69

TABLE V

APPARENT DIGESTIBILITY OF CRUDE PROTEIN

Animal Number		Ladino, Grass Pasture	Sudangrass Pasture	Alfalfa, Orchardgrass Hay	Alfalfa, Grass Pasture
		%	%	%	%
Twin	1	76.32	76.33	61.75	86.29
	2	76.45	74.63	64.70	85.89
Twin	20	49.73*	73.66	55.04	84.23
	21	74.73	74.56	61.00	85.97
Twin	22	63.32	72.47	56.98	83.66
	23	64.52	71.77	52.04	82.23
Non-Twin	6	73.22	71.77	60.68	85.18
	9	73.08	77.72	61.09	86.20
Non-Twin	24	71.79	78.50	56.95	
	25	69.54	77.55	54.12	85.59
	26				83.03
Average		69.27	74.90	58.44	84.83

*This figure believed unreliable due to error in analysis of nitrogen in the feces.

between twin pairs, but the difference found within pairs was not significant. No significance was found between non-twin pairs, but a significant difference ($P < 0.05$) was found within non-twin pairs.

The data concerning the composition of the forage and feces for each ration in this study are given in Tables VI to IX inclusive. The composition of the feces of the identical twins had a smaller variation within pairs than had the non-twin pairs. The between pairs variation was greater for the twin pairs than for the non-twin pairs. The composition follows the same trend as the apparent digestibility data. The units of chromogen and the organic matter seemed to be closely correlated for the twin pairs for all rations except for rations 3 and 4 for twins 22 and 23.

Heat Tolerance

The within pair difference of the three pairs of identical twins in average rectal temperature increase when left one hour in the sun was 0.13° F. and the difference in decrease when taken out of the sun was 0.12° F. On the other hand, the within pair difference of the two non-twin pairs was 0.28° F. in increase and 0.43° F. in decrease in rectal temperatures. The average rectal temperature for the ten heifers before exposure was 101.66° F., after one hour exposure 102.34° F., and after one hour in shade 101.92° F. This was an average increase due to exposure to direct sunlight of 0.65° F. and a decrease of 0.42° F. due to the cooling effects of shade. The range in rectal temperatures for individual observations was from 100.6 to 103.3° F. before exposure, from 101.0 to

TABLE VI

COMPOSITION OF FORAGE AND FECES
RATION 1, LADINO CLOVER, ORCHARDGRASS, FESCUE PASTURE

Animal Number		Dry Matter	Ash	Organic Matter	Crude Protein	Units Chromogen
Twin	1	11.9	2.4	9.5	2.4	35,854
	2	10.1	2.2	7.9	1.9	28,516
Twin	20	6.1	1.3	4.7	2.4	16,894
	21	6.8	1.5	5.3	1.4	19,804
Twin	22	11.5	2.5	9.0	2.4	23,191
	23	11.5	2.6	8.9	2.3	23,180
Non-Twin	6	12.0	2.9	9.1	2.1	28,820
	9	11.4	2.5	8.9	2.0	27,105
Non-Twin	24	12.3	2.4	9.8	2.1	26,775
	25	12.6	2.2	10.4	2.3	26,737
Forage		23.8	2.0	21.8	6.5	23,310

TABLE VII

COMPOSITION OF FORAGE AND FECES
RATION 2, SUDANGRASS PASTURE

Animal Number		Dry Matter	Ash	Organic Matter	Crude Protein	Units Chromogen
Twin	1	9.1	2.2	7.7	2.2	42,607
	2	11.0	2.7	8.3	2.7	49,161
Twin	20	13.5	3.3	10.2	2.6	45,533
	21	10.6	2.4	8.3	2.2	39,284
Twin	22	11.8	3.0	8.8	2.4	39,555
	23	10.1	2.0	8.1	2.3	37,349
Non-Twin	6	13.7	4.1	9.6	2.7	43,298
	9	13.4	5.0	8.4	2.3	46,599
Non-Twin	24	14.0	2.8	11.2	2.8	59,976
	25	12.7	2.8	9.9	2.7	54,969
Forage		21.7	1.8	19.9	4.3	19,766

TABLE VIII

COMPOSITION OF FORAGE AND FECES
RATION 3, ALFALFA, ORCHARDGRASS HAY

Animal Number		Dry Matter	Ash	Organic Matter	Crude Protein	Units Chromogen
Twin	1	12.5	1.2	11.3	1.6	6,969
	2	13.3	1.2	12.1	1.6	7,315
Twin	20	16.5	1.4	15.1	2.2	7,974
	21	16.5	1.3	15.2	2.0	8,549
Twin	22	13.4	1.1	12.3	1.8	7,128
	23	14.1	1.2	12.9	1.9	6,654
Non-Twin	6	15.7	1.2	14.5	1.8	7,692
	9	14.5	1.3	13.1	1.9	8,069
Non-Twin	24	15.7	1.1	14.5	1.9	7,432
	25	13.4	1.1	12.3	1.8	6,703
Forage		88.8	5.8	83.0	14.3	23,817

TABLE IX

COMPOSITION OF FORAGE AND FECES
RATION 4, ALFALFA, GRASS PASTURE

Animal Number		Dry Matter	Ash	Organic Matter	Crude Protein	Units Chromogen
Twin	1	10.6	1.8	8.8	2.1	33,270
	2	7.2	1.4	5.8	1.5	22,751
Twin	20	10.4	1.8	8.7	1.9	25,344
	21	10.9	1.9	9.0	1.9	28,937
Twin	22	10.3	1.7	8.6	2.2	28,642
	23	10.5	2.1	8.4	2.5	30,119
Non-Twin	6	13.9	3.3	10.6	2.6	37,660
	9	12.2	2.3	9.9	1.9	29,104
Non-Twin	25	11.9	2.0	10.0	2.2	32,314
	26	12.4	2.0	10.3	2.7	34,159
Forage		21.7	1.8	19.9	6.2	13,268

104.2° F. after exposure, and 101.0 to 104.4° F. after shade. In one day's measurements twin 22 had the highest observed rectal temperature for each environment. Her mate had rectal temperatures of 103.6° F. after exposure and 103.2° F. after shade the same day. During one other observation the rectal temperatures of this pair were 103.2° and 103.6° F. for 22 and 23 respectively. This same tendency was noticed for the other pairs of twins. Average rectal temperatures and respiration rates are given in Table X.

The average respiration rate before exposure to sunlight was 37.7 per minute, after one hour in direct sunlight 75.1 per minute, and after shade 37.8 per minute. An increase of 37.4 per minute and a decrease of 37.3 per minute in respiration rate was found for the ten heifers. The averages for the individuals compared to their mates indicated that the twins react more nearly alike than do the non-twin pairs. Table XI of the mean and standard deviation of the increase and decrease shows the close resemblance of the twins when compared to the non-twins. The large standard deviations indicated that the animals reacted differently to high temperature stress on different days. This was expected due to the long period over which these observatinns were made and because of the variations in ambient temperature.

The average rectal temperatures and respiration rates for the three pairs of identical twins on different levels of nutrition are presented in Table XII. The average rectal temperature of the group on the high grain ration was 102.70° F. and that of the group on the normal grain ration was 102.30°.F. The average respiration rates of the two groups

TABLE X

RECTAL TEMPERATURE AND RESPIRATION RATE
(Average of Ten Observations)

Animal Number		Rectal Temperature			Respiration Rate		
		Inside	After Exposure	Shade 1 Hour	Inside	After Exposure	Shade 1 Hour
Air Temp.		77.7	85.9	80.2			
Twin	1	101.60	101.93	101.78	30.2	66.7	25.5
	2	101.70	102.06	101.73	28.5	65.8	25.2
Twin	20	101.62	102.14	101.80	35.3	79.7	37.8
	21	101.84	102.58	102.10	35.0	79.1	39.6
Twin	22	102.09	102.69	102.35	51.0	94.9	49.0
	23	101.96	102.67	102.28	56.2	97.0	52.6
Non-Twin	6	101.31	101.68	101.56	34.8	54.4	31.1
	9	101.17	101.81	101.26	35.1	64.6	31.0
Non-Twin	24*	101.70	103.09	102.16	27.89	71.67	42.0
	25	101.62	102.72	102.21	43.0	77.0	43.8
Average		101.66	102.34	101.92	37.7	75.1	37.8

*Average of nine observations.

TABLE XI

MEAN AND STANDARD DEVIATION OF THE INCREASE AND DECREASE IN RESPIRATION

Animal Number		Increase Mean	Increase Standard Deviation	Decrease Mean	Decrease Standard Deviation
Twin	1	36.5	13.51	41.2	16.79
	2	37.3	9.60	40.6	12.50
Twin	20	44.4	20.33	41.9	20.64
	21	44.1	15.90	39.5	19.92
Twin	22	43.9	18.80	45.9	19.77
	23	40.8	18.42	44.4	22.68
Non-Twin	6	19.6	11.61	23.3	12.44
	9	29.5	14.60	33.6	13.44
Non-Twin	24*	42.1	19.13	29.9	21.35
	25	34.0	14.14	33.2	19.03

*One observation calculated for non-twin 24.

TABLE XII

RECTAL TEMPERATURE AND RESPIRATION RATE
(Average of Four Observations)

Animal Number	Feeding Level	Rectal Temperature	Respiration Rate
Twin 10	High	102.38	73.5
11	Low	102.03	59.0
Twin 12	Low	102.08	64.0
13	High	102.78	81.5
Twin 14	Low	102.68	63.0
15	High	102.93	66.3

were 77.08 per minute and 62.0 per minute for the high and normal grain rations respectively. The effect of feeding level on rectal temperature was less than the difference between pairs. The greatest within pair difference occurred in twin pair 12 and 13, which were grade Holsteins. Respiration rates were highest for twins 10 and 11, while the rectal temperatures were lowest for this pair. In the case of twins 14 and 15 the reverse was found. Of the group on high grain feeding, condition of the animals from highest to lowest was for twins 15, 13 and 10 in that order.

Growth

Growth of the identical twin heifers, as measured by body weight, heart girth, and withers height is shown by smoothed curves in Figures 1 to 6. The within pair growth rates of twin pairs 1 and 2, 20 and 21, and 22 and 23 were very similar. Twins 20 and 21 lost weight during the digestion trials while twins 22 and 23 increased in weight at a decreased rate. Twin pair 22 and 23 appeared to be more hardy than the other two pairs. The growth curves of the pairs on different levels of nutrition showed a different rate of gain as measured by body weight. Pair 14 and 15 had a larger spread in weight than the other two pairs. As expected, a greater difference was found between pairs than within pairs. In condition there was little difference within the pairs fed at the same level. The animals on the different levels of feeding were easily distinguished by the difference in condition a few weeks after the start of the experiment.

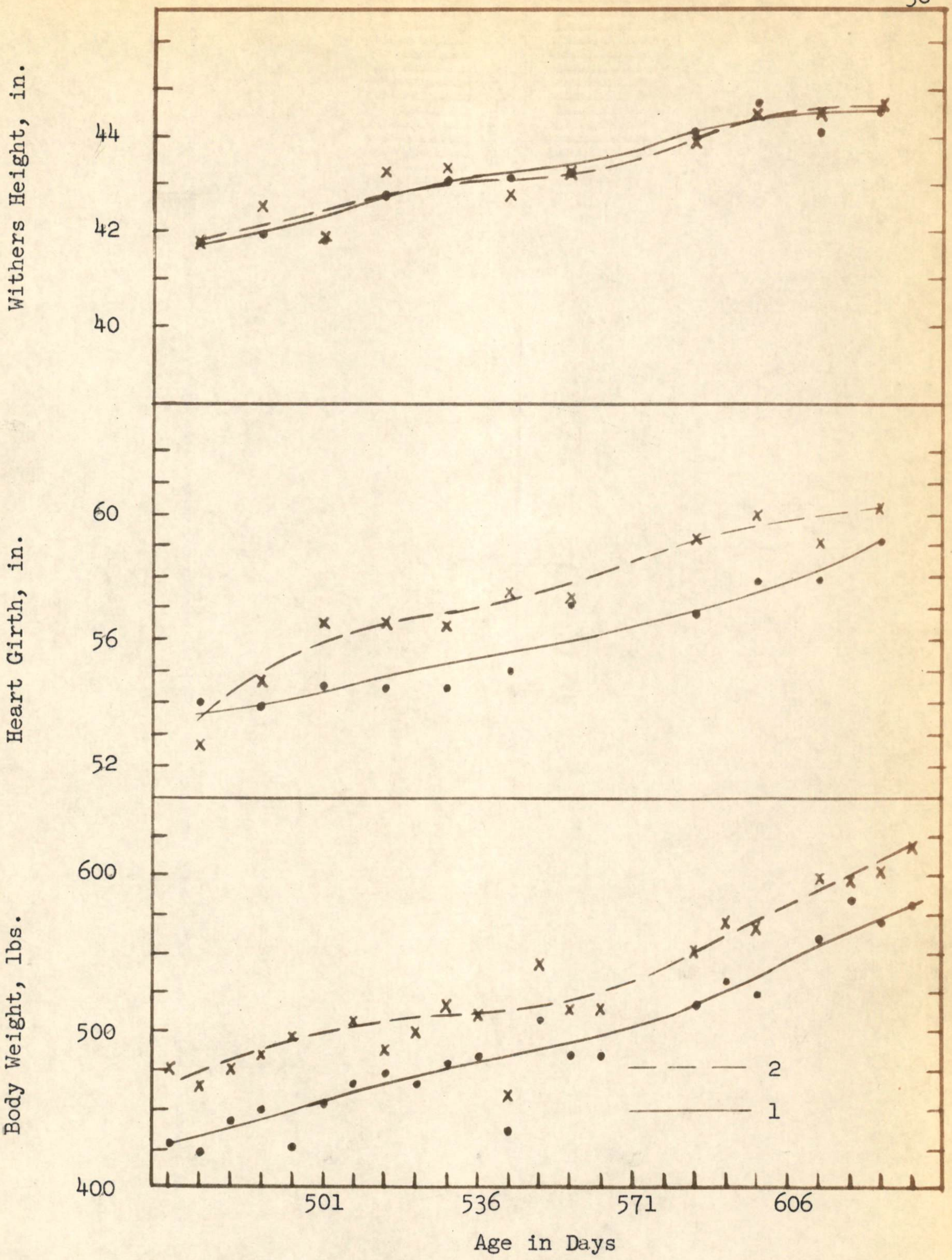

Figure 1. Growth rates of Twins 1 and 2

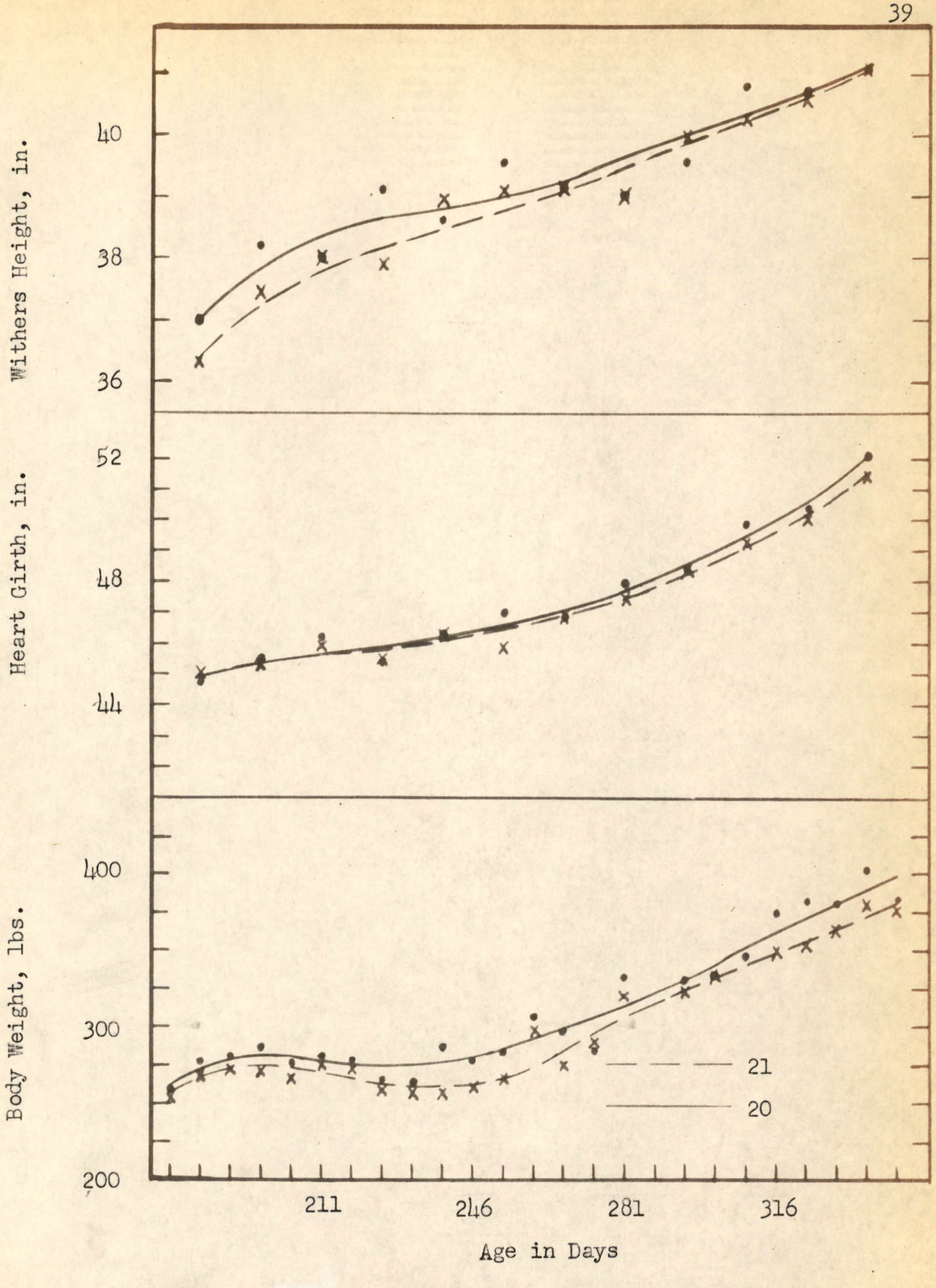

Figure 2. Growth rates of Twins 20 and 21

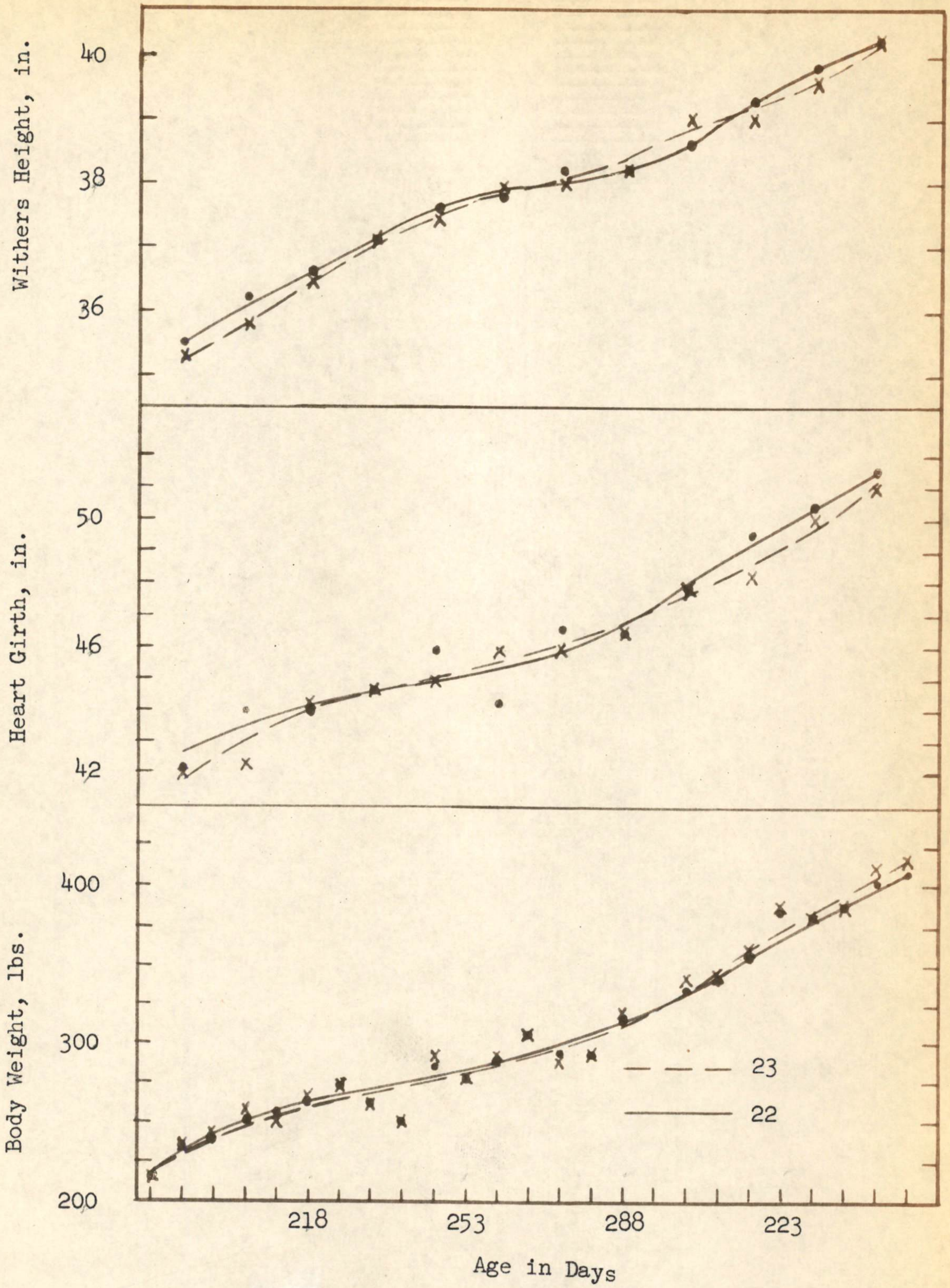

Figure 3. Growth rates of Twins 22 and 23

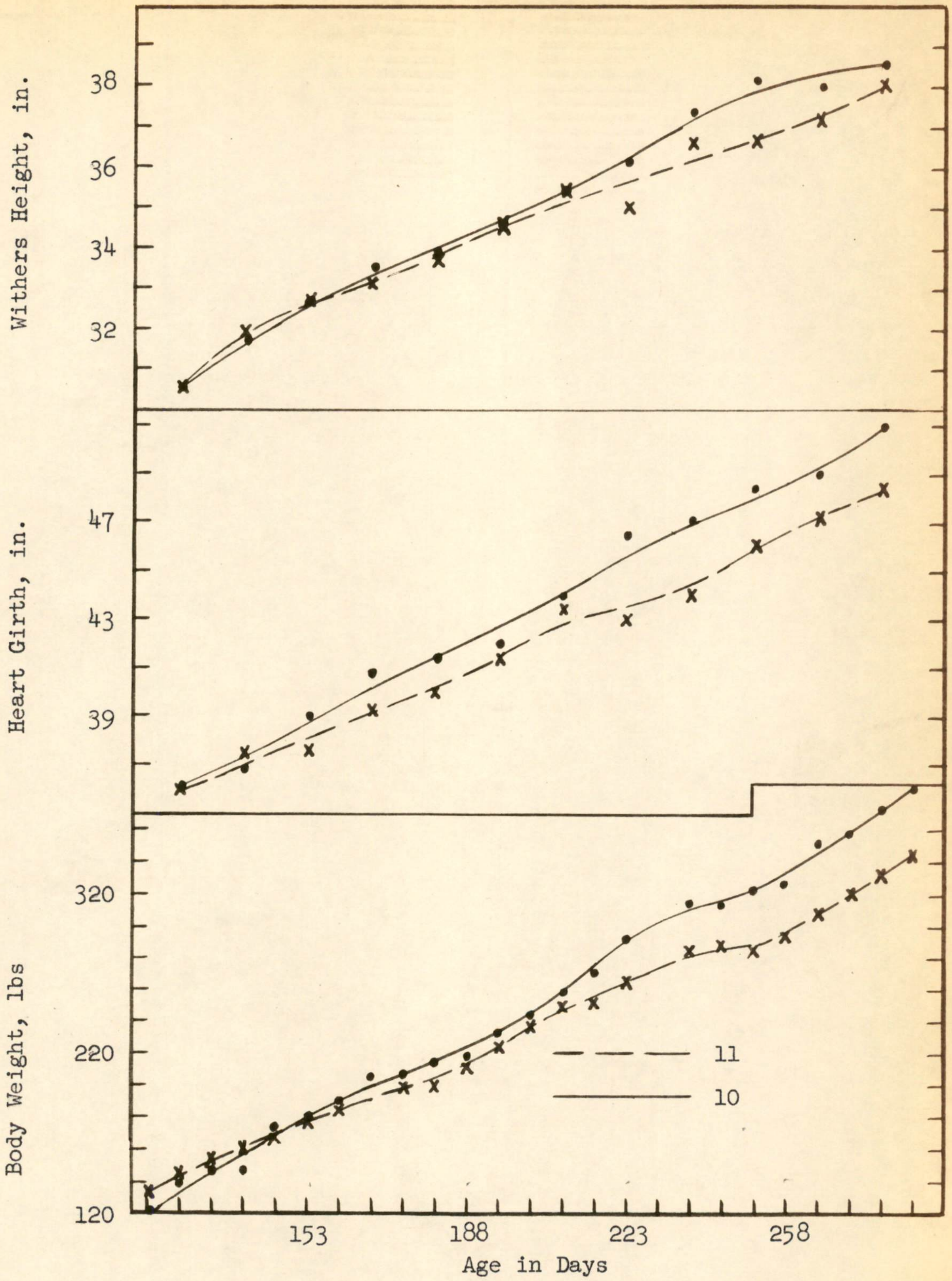

Figure 4. Growth rates of Twins 10 and 11

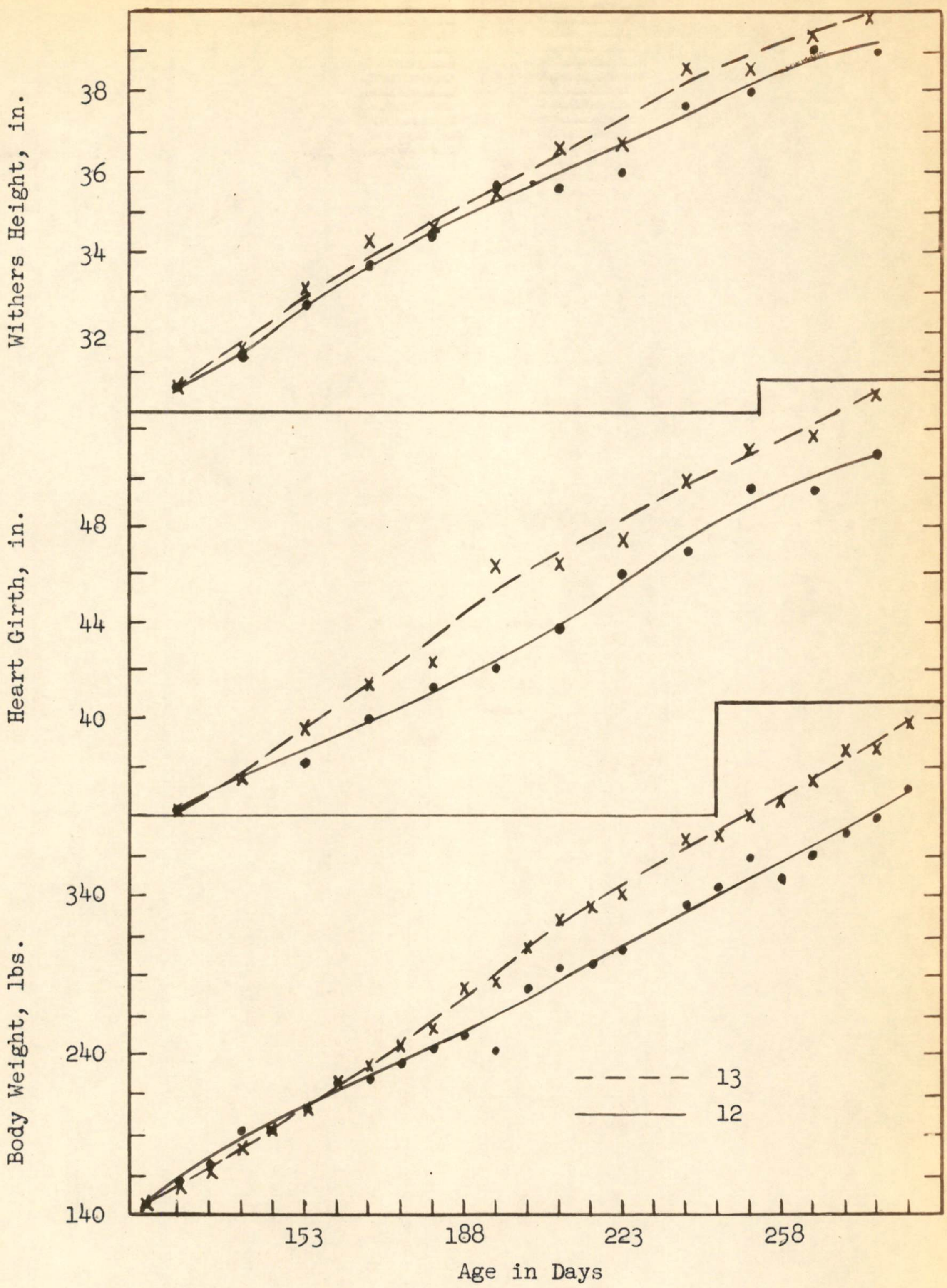

Figure 5. Growth rates of Twins 12 and 13

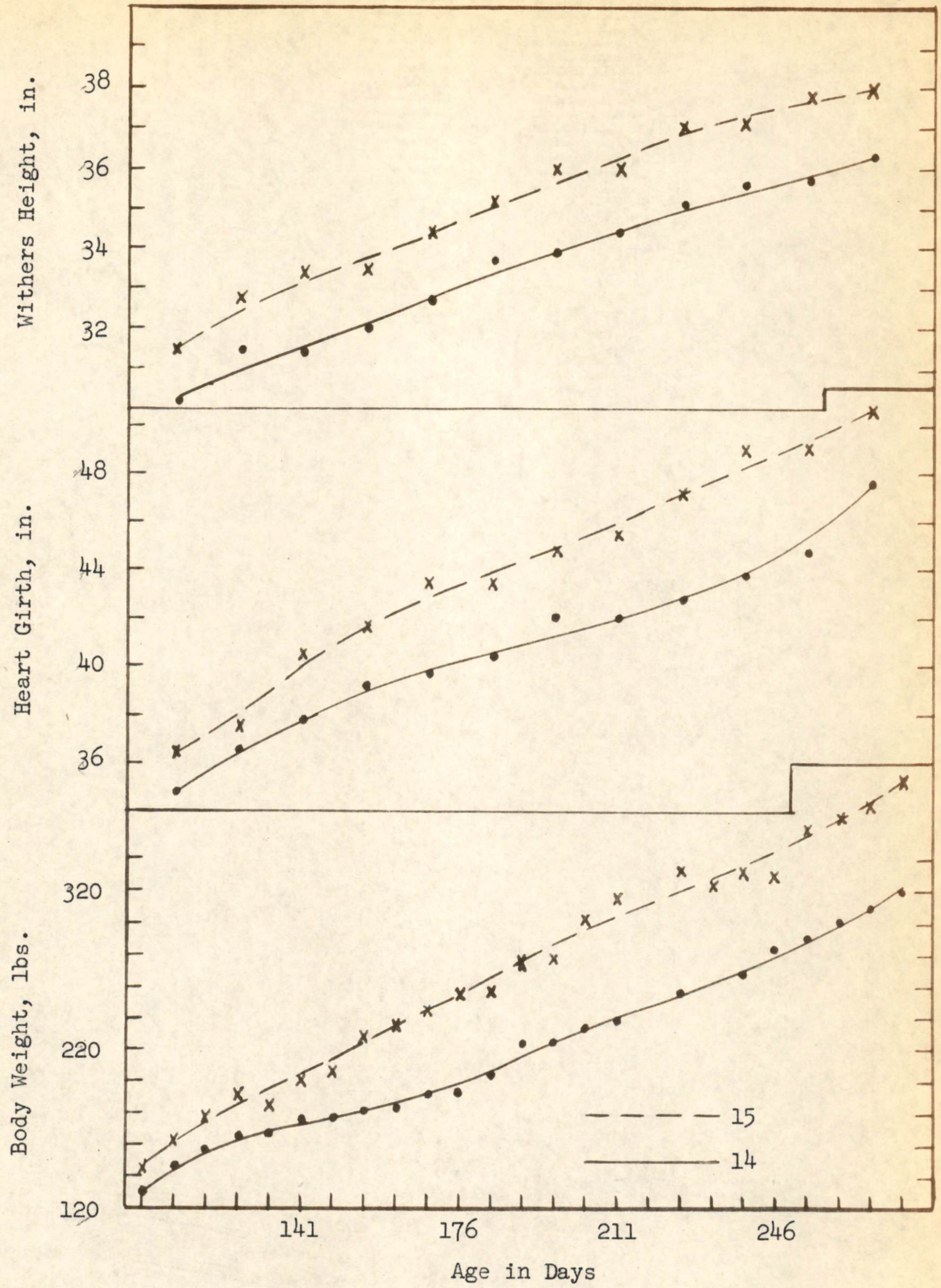

Figure 6. Growth rates of Twins 14 and 15

The three pairs on equal feeding levels were below the Ragsdale (49) standard in all measurements. Twins 20 and 21 and 22 and 23 were fast approaching the standard in body weight and heart girth measurements at the end of the study. The withers heights of these pairs were below normal. Twins 1 and 2 had measurements below the normal. These animals were older than the other pairs when purchased and the feeding level before the Experiment Station obtained this pair was not very high. The normally fed animals in the pairs on different levels of feeding were below normal in all cases. Only twin 15 of the high grain group was above the normal growth curves in body weight and heart girth. Twin 10 had attained normal weight and heart girth at the end of the experiment. Twins 12 and 13 grew at about normal rates but remained subnormal to the standard for Holsteins in all measurements. Since all of these calves were much below normal in size at birth, the early growth data were not expected to approach normal figures.

DISCUSSION

Averages and ranges in the apparent digestion coefficients observed for the monozygous twins and the selected pairs of heifers agree with those of Reid et al. (52) and McCullough et al. (46) for pasture forage. Since the twin pairs showed a closely related ability to digest forage to the same degree of efficiency, the writer must conclude that factors influencing digestive capacity are inherited. Twins on pasture reacted quite similarly. The consistence of the feces seemed to be the same, and the dry matter content of the feces (Tables VI-IX) proved this observation to be accurate. Twins 1 and 2 had a more fluid feces throughout the experiment than did the other animals. On ration 1 twins 20 and 21 scoured profusely during the whole period while the other animals were not seriously affected. Twin 21 had recovered some on the day of collection of feces. A reason for the differences found in the digestibility by the twins in the pastured rations could be that differences in the forage eaten influenced the digestibility coefficients. Identical twins were very much alike in grazing. Twins 20 and 21 were observed to select the tops of the plants while twins 22 and 23 ate most of the plants to the ground. Twins 1 and 2 grazed less than the other animals while an observer was in the field. Pairs of twins stayed together except when one was in estrus. Hancock (28) found the same characteristics for grazing twins. Considering age, twins 1 and 2 and non-twin 25 appeared to be in poorer condition of flesh than the other animals. The non-twins were from the same herd of cattle, which adds to the information pointing to the inheritance of factors influencing digestive capacity. The differences in digestion coefficients between pairs of identical twins were much greater than the difference between non-twin pairs. For this reason use of monozygotic animals in digestion studies would give a more accurate picture of the variations between animals from widely dispersed herds in the section from which the twins are collected. The effects of two treatments upon the digestibility of forage could be determined at the same time, thereby eliminating the error due to time.

The effects of sunlight upon the rectal temperatures and respiration rates (Table X) closely agree with Seath and Miller (60, 62) and Gaalaas (22). The small variation in means of the increase and decrease (Table XI) in respiration within pairs of twins as compared to non-twin pairs and between pairs of twins clearly indicated that the heat tolerance of monozygous twins is identical. The animals on a high grain ration had higher body temperatures and respiration rates than did their mates on normal rations. This agrees with Robinson and Lee (57). Outward reactions of twins under heat stress were identical. Twins 20 and 21 threw bedding with their front feet. Twins lay down about the same time, while the non-twins did not show this characteristic. Of the three pairs of twins on high and normal grain rations, each pair had a characteristic not exhibited by another pair. Twins 10 and 11 splashed water, twins 12 and 13 lay in wet bedding, and twins 14 and 15 drank frequently when ambient temperature was high. The twins on the high grain ration started these cooling methods at lower temperatures than did their sisters on normal feed. Evidently heat tolerance is inherited. Monozygous twins are ideal subjects for studies comparing the effects of different temperature environments upon body reactions.

Monozygotic heifers on the same feed grew at nearly identical rates, with only twins 1 and 2 showing a difference of more than a few pounds in weight. This pair had an almost constant difference for the whole period. Twins 20 and 21 were not in as good condition of flesh as twins 22 and 23, although both pairs gained weight at about the same rate except for the period when an all roughage ration was fed. Twins 20 and 21 failed to gain during this period, while the rate of gain was slowed down for twins 22 and 23. The high grain ration caused an increase in the growth rate of calves compared to their identical mates fed a normal ration. A greater difference in rate of growth is shown between non-related animals on the same ration than between identical twins fed different rations. These results are in accord with those of Bonnier and Hansson (6), Bonnier, Hansson and Skjervold (9).

Combining the results indicated that factors influencing digestibility of forage, heat tolerance, and growth are all inherited. Monozygotic animals are ideal for experimental comparisons to determine the digestibility of different forages, and at the same time determine the effects of the different forages upon heat tolerance and growth.

SUMMARY

Three pairs of monozygotic dairy heifers and two pairs of selected heifers were compared in two physiological studies, digestion of forage and heat tolerance. Statistical analyses of the data on digestion presented showed a highly significant difference ($P < 0.01$) between pairs of identical twins for both dry matter and protein, but the differences within twin pairs were not great enough to be significant. A significant difference within pairs of selected non-twin animals was found for protein digestion coefficients, but not for dry matter. No significant differences in dry matter or protein digestibility were found between pairs of non-twins. The heat tolerance of the animals as measured by rectal temperatures and respiration rates indicated that identical twins inherit the same heat tolerance in like environments. Little difference was found within identical pairs. A large difference between pairs of twins and within pairs of selected animals was noted. Smoothed growth curves were nearly identical for monozygous animals fed the same ration.

Identical animals fed a high grain ration grew faster than their mates on a normal ration, but the growth curves of twins were more alike than for non-related animals on the same feed. The twins fed unlike rations reacted differently in the heat tolerance tests.

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