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THE EFFECT OF A 24-HOUR FAST AND GLUCOSE-FEEDING ON THE WEIGHT AND COMPOSITION OF THE LIVER IN SUCKING CALVES

By

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It has long been known that transport and/or starvation for a short time before slaughter result in considerable losses in the weight of carcass as well as of various organs, notably the liver. The composition of the liver alters, in that, for instance, the water and glycogen contents diminish. The weight-losses and the changes taking place in the liver can be reduced or eliminated by feeding before slaughter, but only if the diet contains sugar (*Schön & Scheper 1957*). These observations have been made almost exclusively on pigs (*Bjorka 1938, Callow et al. 1939, Gibbons & Rose 1950, Otto & Steger 1963, Saffle & Cole 1960, and others*). Investigations into such effects in cattle are very few. *Howard & Lawrie (1956, 1957)* and *Hedrick et al. (1957)* found that the glycogen content in the liver of cattle is not affected by exercise. *Greenwood et al. (1953)* and *Wilcox et al. (1953)* noted that oral administration of sucrose before slaughter increased the weight and carbohydrate content of the liver in beef cattle. According to *Cazaillet (1962)*, the carcass weight and the liver weight in calves decrease greatly when they are fasted for 3 days before slaughter and this reduction can be largely eliminated by giving the calves food containing milk and sugar.

The losses in weight of the calf liver and its glycogen content do not only raise problems concerning food hygiene and economy but are also of clinical interest, with a view to the young calves that are sold for further rearing. In 1964, about 13 % of the calves born in Sweden were slaughtered as sucking calves, while

8 %, or 80,000, of the sucking calves were sold through the slaughter organisation for further rearing. Under the system applied to the sale of these calves in Sweden, 24 hours will in most cases elapse from the time the calf has left the seller until it reaches the buyer. The feeding of the calves during this interval involves some difficulties, the greatest problem being that at the change-over to milk substitutes the calves often develop diarrhoea. Glucose solution, on the other hand, has no adverse effects on the digestive system. From various viewpoints it is therefore of interest to find out to what extent a 24-hour fast will affect the glycogen content of the liver and whether a reduction of glycogen, if any, can be off-set by feeding glucose.

MATERIAL AND METHODS

In 1964: 33 sucking calves and 32 fattening calves were slaughtered, 15 and 16, respectively, on the day of transport and 18 and 16, respectively, on the next day. The latter had no access to water and food from their arrival at the slaughter-house and up to the time of slaughter. The chilled carcass weight and the liver weight were measured in a precision of ± 0.05 kg and ± 0.005 kg, respectively.

The relative water content of the liver was determined in 7 of the calves slaughtered on the day of transport and in 10 of those slaughtered on the next day.

Table 1. Times of slaughter and treatment of the calves between end of transport and slaughter.

Group	n	Time of slaughter after end of transport	Treatment
I	5 ¹⁾	within 1½ hours	—
II	5	20—23 hours	Fasting
III	5	20—23 hours	Oral administration of 2.5 litres of 5 % glucose solution per calf 18 and 2 hours before slaughter

¹⁾ On inspection of the carcasses the fifth calf was found to have acute purulent gonitis and was therefore excluded from the study, as the disease had very probably affected its general condition and appetite. This was also confirmed by the liver analyses (Table 6).

In 1965: 15 sucking calves about 14 days old, including 14 males, were weighed and transported about 100 kilometres. They were divided by weight into three equally large groups. The first group was slaughtered within 1½ hours of the end of the transport. The other two groups were slaughtered on the next day, starting 20 hours after the end of the transport. The slaughter was completed 3 hours later. The calves in the second group were given neither water nor food. Those in the third group were each given 2.5 litres of 5 % glucose solution per os 18 and 2 hours before the beginning of slaughter. Data on the three groups are set out in Table 1. For the calves slaughtered on the second day, the order was as follows: First calf no. 1 in group 2, then calf no. 1 in group 3, thereafter calf no. 2 in group 2, and so forth.

The liver was removed as quickly as possible and weighed in a precision of ± 5 g; specimens for chemical analyses were taken from the lobus sinister and for histological examination from the lobus sinister and lobus caudatus. The specimens for the chemical analyses were weighed in a precision of ± 0.5 mg. The time interval between the moment of killing and the beginning of the glycogen determination (*vide infra*) was kept as constant as possible. It varied from 15 minutes and 5 seconds to 15 minutes and 50 seconds. The warm carcass weight was recorded in a precision of ± 0.05 kg.

Chemical analyses

The liver was analysed for water, fat, crude protein, and glycogen by the following methods:

Water: Gravimetric determination. The specimen (about 15 g) was finely ground with ignited sea sand and dried to constant weight in thermostat at 103°C.

Fat: Extraction in ether for 16 hours from a liver specimen weighing about 15 g, using a Soxhlet's apparatus.

Crude protein: By Kjeldahl's method on a specimen weighing about 1 g.

The water, fat, and crude-protein determinations were made in duplicates.

Glycogen:

1. Isolation of glycogen from the liver.

A specimen weighing about 10 g was introduced into a 100-ml centrifugal tube of glass, together with 30 ml of 30 % KOH heated in water-bath to 98—100°C. The tissue was dissolved by heating in a briskly boiling water-bath for exactly 25 minutes. After cool-

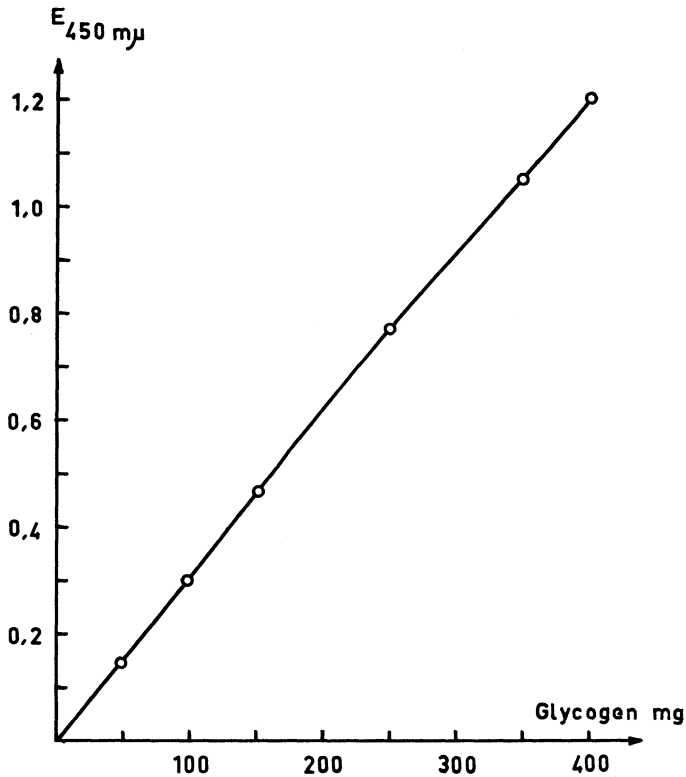


Figure 1. Standard curve representing known amounts of glycogen.

ing for 1 minute at room temperature, the glycogen was precipitated with 1.2 volume of ethanol. After cooling to room temperature, the glycogen was washed twice in ethanol. It was then dried and the dry weight was determined approximately by weighing.

2. Quantitative determination of glycogen.

To assure an accurate measure of the glycogen content, a method was worked out which is based on an enzymatic determination of glucose in body fluids described by *Levin & Linde* (1962). The glycogen was hydrolysed in 30 ml of 0.5 M- H_2SO_4 in a boiling water-bath for exactly 2½ hours. The solution was filtered and diluted to 50, 100, 200, 400, or 500 ml, according to the result of the gravimetric determination of glycogen. The glucose in the hydrolysate was determined in duplicate enzymatically by means of spectrophotometry. The extinction was read in a Beckman-B spectrophotometer at the wave-length of 450 mμ. A standard curve was constructed from the values obtained by hydrolysis of known amounts of glycogen p.a. and the subsequent enzymatic determination of glucose in the hydrolysate diluted to 100 ml. The standard curves for the rest of the dilutions coincided after recalculation to 100 ml with the curve shown in Fig. 1. Triplicate

Table 2. Variation obtained at triplicate glycogen determinations.

Variation	d. f.	Sum of squares	Variance	F
Between calves	10	247.2012	24.7201	279.3***
Within calves (error)	22	1.9459	0.0885	
Total	32	249.1471		

glycogen determinations were made on each liver, except from three calves on whose livers, because of unfortunate accidents, only one determination was made in 1 case and only two in 2 cases.

3. Variations in the glycogen determinations.

The glycogen in the liver is unevenly distributed (*Otto & Steger 1963*), and therefore the result of repeated glycogen determinations will vary between specimens from one and the same liver. The post-mortal glycolysis alters the glycogen content quickly. In the present investigation, the latter variation between different determinations was, as far as can be done, eliminated by the strict standardisation of the treatment of the specimens and the analytical procedure. Variations also occur between the determinations because of the error of the method of analysis. The sum of these causes of variation, the total error, in triplicate determinations, will be seen in Table 2. The variation between calves was highly significantly greater than within calves (= between repeated determinations). Accordingly, the technique used for collection of specimens and glycogen analysis was sufficiently accurate to establish with high degree of certainty differences in the glycogen content between the livers of different calves. In view of this, the single determination in one calf and the duplicate determinations in two calves were also included.

Table 3. The carcass weights, absolute and relative liver weights of sucking calves and fattening calves, when slaughtered immediately after end of transport (A) or on the next day (B).

	Carcass wt. $\bar{x} \pm s$, kg	Liver wt. $\bar{x} \pm s$, kg	Rel. wt., liver $\bar{x} \pm s$, %
Sucking calves			
A n = 15	24.5 \pm 2.8	0.88 \pm 0.22	3.57 \pm 0.74
B n = 18	23.7 \pm 4.2	0.63 \pm 0.13	2.66 \pm 0.47
t-value	< 1	4.14***	4.25***
Fattening calves			
A n = 16	62.0 \pm 19.9	1.94 \pm 0.63	3.14 \pm 0.41
B n = 16	56.1 \pm 12.8	1.52 \pm 0.35	2.70 \pm 0.26
t-value	1.00	2.33*	3.58**

Histological examinations

The specimens for histological examination were fixed in 10 % formalin solution and in Carnoy's solution. The formalin-fixed specimens were cut in frozen sections into a thickness of 10—15 μ and stained with Sudan III. The Carnoy-fixed specimens were embedded in paraffin, cut into a thickness of about 5 μ and stained in periodic acid-Schiff (PAS).

Statistical methods and symbols

Before the chemical and histological examination, each sample was marked with a random number and the examinations were then carried out in sequence according to these numbers.

Analysis of variance and Student's t-test were used in the statistical calculations.

The following system was used to indicate the level of significance:

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. The abbreviations used were:

- n = number of values
- d.f. = degrees of freedom
- \bar{x} = mean
- s = standard deviation
- F = variance ratio

RESULTS

1964, preliminary observations. The results of the preliminary studies are shown in Tables 3 and 4. In sucking calves as well as in fattening calves which were slaughtered on the day after the transport to the slaughter-house, both the absolute and the relative weight of the liver were significantly lower than in calves slaughtered immediately after the transport. The average reduction in liver weight was about 28 % for sucking calves and about 22 % for fattening calves. There was no significant difference in the carcass weight or the water content of the liver between the groups.

Table 4. Water content of livers from sucking calves (groups A and B according to Table 3).

	A n=7	B n=10
Water content, %		
$\bar{x} \pm s$	72.5 \pm 1.9	73.8 \pm 1.5
	t < 1	

Table 5. Live-weight, carcass weight, absolute and relative liver weight.

Group	I Control n=4	II Fasted n=5	III Glucose n=5	t-value at comparison		
				I-II	I-III	II-III
Live-weight,						
kg						
$\bar{x} \pm s$	40.0 \pm 3.4	39.2 \pm 4.0	40.0 \pm 1.9			
Range	38.0—45.0	34.0—45.0	38.0—43.0			
Carcass wt.,						
kg						
$\bar{x} \pm s$	23.0 \pm 3.0	23.1 \pm 2.9	23.9 \pm 1.7			
Range	19.8—27.1	19.7—27.7	21.8—26.4			
Liver wt.,						
g						
$\bar{x} \pm s$	855 \pm 93	652 \pm 86	712 \pm 88	3.41*	2.36	1.09
Range	730—930	530—770	610—850			
Rel. liver						
wt., (% of						
carcass wt.)						
$\bar{x} \pm s$	3.73 \pm 0.50	2.82 \pm 0.13	2.98 \pm 0.31	3.65**	2.76*	1.06
Range	3.23—4.24	2.69—3.04	2.46—3.22			

1965. It will be seen from Table 5 that there were no differences between the three groups in live-weight or carcass weight. Consequently, there were no differences in dressing percentage either.

The liver weight was almost significantly higher in the control group than in the fasted group, whereas differences between the control group and the glucose-fed group were not significant ($0.10 > P > 0.05$). Corresponding differences between the groups were found in the relative weight of the liver; in this respect the two experimental groups deviated significantly from the control group. The difference in the average absolute weight of the liver between the control group and the fasted group, 203 g, constitutes about 24 % of the average liver weight of the control group.

The percentage contents of glycogen, fat, crude protein, and water are set out in Table 6. In the fasted calves the proportions of glycogen were lower but those of fat, crude protein, and water higher than in the control calves. All the differences were significant. The glucose-fed calves showed intermediate values in every respect, except for the content of water.

Table 6. Liver content of glycogen, fat, crude protein, and water, as percentages.

Group	I Control n=4 ¹⁾	II Fasted n=5	III Glucose n=5	t-value at comparison		
				I-II	I-III	II-III
Glycogen						
$\bar{x} \pm s$	6.45 \pm 0.76	0.20 \pm 0.12	2.99 \pm 1.48	18.5***	4.22**	4.21**
Range	5.61—7.33	0.05—0.33	1.47—4.62			
Fat						
$\bar{x} \pm s$	1.13 \pm 0.21	2.78 \pm 0.29	1.72 \pm 0.50	9.34***	2.20	4.10**
Range	0.93—1.28	2.35—3.15	1.27—2.39			
Crude protein						
$\bar{x} \pm s$	18.3 \pm 0.5	21.8 \pm 0.4	19.1 \pm 1.5	10.7***	1.05	3.85**
Range	17.5—18.7	21.5—22.5	17.4—20.9			
Water						
$\bar{x} \pm s$	73.2 \pm 0.6	74.6 \pm 0.4	75.4 \pm 1.6	4.25**	2.63*	1.11
Range	72.4—73.7	73.9—74.9	74.3—78.1			

1) The fifth calf, which was excluded from the study because of acute purulent gonitis, showed the following values: glycogen 1.08, fat 1.23, crude protein 23.2, water 73.5.

As the liver weights differed between the groups, the differences in the proportions of the various substances are not always paralleled by similar differences in the total amounts. Thus, as will be seen from Table 7, both the fasted and the glucose-fed group showed smaller amounts of glycogen, crude protein, and water and larger amounts of fat than did the control group. In this respect, too, the glucose-fed group occupied an intermediate position. The changes in the glycogen content were verified by the histological examination.

The differences in the average liver weights and in the average total amounts of glycogen, fat, crude protein, and water between the groups are shown in Table 8. The lower absolute liver weights in the fasted and the glucose-fed groups were to about 70 % and 63 %, respectively, due to a reduced content of water and to about 26 % and 24 %, respectively, to a reduced content of glycogen. The fat content, on the other hand, was increased in both groups, which was also verified histologically. Histochemically demonstrable fat was present in only one calf in the control group, whereas all the calves in the other two groups had finely globular,

Table 7. Liver content of glycogen, fat, crude protein, and water, total amounts in g.

Group	Control n=4	Fasted n=5	Glucose n=5
Glycogen			
Range	47.1—68.2	0.3—2.2	12.5—33.3
\bar{x}	55.2	1.3	21.2
Fat			
Range	7.3 —12.1	12.2—21.6	9.4—14.9
\bar{x}	9.7	18.0	12.0
Crude protein			
Range	135 — 170	114 — 165	123 — 154
\bar{x}	156.3	140.0	135.4
Water			
Range	529 — 684	396 — 577	458 — 664
\bar{x}	626	480	537

often diffuse, fatty degeneration of the liver cells, described as slight in the fasted group and negligible to slight in the glucose-fed group.

DISCUSSION AND CONCLUSIONS

A considerable reduction of the liver weight was noted within only 24 hours of fasting. It was of the same order of magnitude as that of 30 % reported by *Cazaillet* (1962) after 72 hours of fasting. According to *Cazaillet*, fasting for such a long period leads to a reduction of the carcass weight by 7 %. The carcass weight was not influenced by a shorter fasting period in our study.

Table 8. Differences in average liver weights and average total amounts of water, glycogen, crude protein, and fat between control group and fasted group, and between control group and glucose-fed group.

Groups	Control v. fasted		Control v. glucose-fed	
	Difference g	Per cent of total difference	Difference g	Per cent of total difference
Liver weight	203		143	
Water	— 146	70.2	— 89	62.7
Glycogen	— 53.9	25.9	— 34.0	23.9
Crude protein	— 16.3	7.8	— 20.9	14.1
Fat	+ 8.3	4.0	+ 2.3	1.6

The liver weight seems to be more readily affected by fasting in calves than in swine. *Callow et al.* (1939) found that the weight of the liver was reduced by only about 12 %, when pigs before slaughter were rested and not fed overnight. This reduction could be wholly eliminated by one feed of 2 lb of mixed meal. According to *Saffle & Cole's* (1960) results, the liver weight of swine fell by about 5 % in the first 24 hours of fasting and not until the end of 96 hours was it 26 %, or of the same order of magnitude as that noted in the 24-hour fasted calves in our study. The difference between calves and swine in this respect may be due to the effect of age and need not signify a difference between species.

To what extent the weight reduction is due to losses of water and/or to losses of dry substances is not known, but it is believed that the weight losses represent mainly water (*Ingram* 1964). From investigations on fatigued pigs there are indications that the moisture content of the liver is lower than usual (*Lewis et al.* 1961). In our investigated calves a 24-hour fast did not cause any reduction of the water content of the liver, calculated as a percentage. On the contrary, the water content was higher both in the fasted and in the glucose-fed calves than in the control animals. The lower weight of the liver in the two experimental groups means, however, that the absolute amount of water was so greatly reduced that the loss of water comprised the main part of the weight-loss. But the decrease of liver glycogen was probably the primary effect and the loss of water a manifestation of the body's effort to maintain a constant water content in the tissues.

It is obvious that the liver glycogen, being a readily available source of energy, is utilised during starvation. *Rose & Peterson* (1951), in experiments on rats, found that a 24-hour starvation period depleted the liver glycogen. The degree to which the liver glycogen decreases during a short period of starvation in calves does not seem to have been studied earlier. *Cazaillet* states that calves are poorly resistant to transport stress. It is apparent from the present investigation that this lack of resistance can in part be due to the fact that the liver glycogen is broken down very quickly. It had disappeared almost completely in 24 hours. An increased incretion of catecholamines may have been a factor concerned in the rapid breakdown of the glycogen. The observed fatty degeneration of the liver is supporting evidence for this pre-

sumption (*Wirsén* 1965). The loss of crude protein suggests that metabolic pathways other than the carbohydrate metabolism had been increasingly used.

The rise in liver glycogen and a restoration of the liver weight after feeding have been repeatedly observed. *Wilcox et al.* (1953) gave beef cattle 6 lb of sucrose 30 hours before slaughter and found that the carbohydrate content of the liver, calculated as dextrose, increased from 1.66 to 1.92 % in steers and from 1.40 to 1.85 % in heifers. The liver weight rose from 12.2 to 15.8 lb in steers but showed a tendency to decrease in heifers. An increase of the amount of sucrose from 6 to 12 lb produced no further effect. *Cazaillet* states that the 30 % reduction of the liver weight of calves starved for 72 hours can be brought down to 8 % by supplementing food containing milk and sugar.

Numerous observations in swine show that feeding affects the weight and the glycogen content of the liver in the same way and to a similar degree (*Callow et al.*; *Gibbons & Rose* 1950, and others).

As stated by *Ingram*, in most of these experiments sugar has been fed together with ordinary food, so that it is not easy to recognise the specific effect of sugar.

In the present investigation, oral administration of 2.5 litres of 5 % glucose solution at 18 and 2 hours before slaughter did not fully compensate for the losses in weight and glycogen content of the liver. The glycogen content was, however, significantly higher in glucose-fed than in fasted calves.

The loss of liver glycogen will in all probability lower the resistance of the calves to various forms of stress. A lowering of their resistance to infections cannot be excluded. From the viewpoint of food hygiene this is an important consideration, because of the risk of cross-infection in the slaughter-house stalls, where animals from different environments are brought together. Being in a very acute stage, any such infection would be very difficult to discover both at the ante-mortem inspection of the calf and at the post-mortem inspection of the carcass.

Usually, calves are slaughtered on the day after arrival at the slaughter-house. It is generally considered that this "resting period" improves the quality of the meat and, hence, the economical value of the product. For the reasons discussed in the foregoing, we think that hygienically as well as economically it would be preferable to slaughter the calves as soon as possible after arrival at the slaughter-house.

As regards calves that are sold as described in the beginning of this paper, it is important that the liver should not be deprived of its glycogen, as this would in all probability lower their resistance to the stress of transport and changes of environment. The question whether more complete restoration can be achieved only by increasing the glucose supply more than what was done in our cases, or whether other food substances must be used, has to be investigated. For calves sold for further rearing, on the other hand, it would certainly be better to attach less importance to the maintenance of glycogen concentration than to add milk substitutes to their glucose supply, with the risk of causing diarrhoea. The amount of glucose may, however, be increased from 250 g used in this study to 500 g per day in the same amount of water, at least until further investigations have shown the most suitable amount of glucose to be given.

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SUMMARY

Fifteen calves were divided by body-weight into three groups of 5 animals in each. One of the calves was found after slaughter to have had purulent gonitis and was therefore excluded from the study. One group, the control group, was slaughtered immediately and the other two groups 20 hours after the end of the transport. One of the latter two groups received neither water nor food during this period and the other was given a total of 250 g of glucose as 5 % solution on two occasions. The carcass weight and the liver weight were recorded and the liver was analysed for its content of water, glycogen, fat, and crude protein.

The liver weight was significantly lower in the calves of the fasted group than in the control animals. The loss in weight of the liver was due mainly to water depletion, although the proportion of water in the livers of the fasted calves was higher than in the livers of the control animals. The starvation resulted in almost complete disappearance of the liver glycogen and, also calculated as a total, a decrease of the amount but an increase of the proportion of crude protein. The amount of fat in the liver was increased, which was attributable to slight diffuse, globular, fatty degeneration of the liver cells. The glucose-fed group showed intermediate values in relation to the fasted and the control group in almost every respect. The importance of the results from the viewpoint of food hygiene as well as their clinical significance are discussed with references to the relevant literature.

ZUSAMMENFASSUNG

Die Einwirkung des eintägigen Hungern und der Glukosezufuhr auf das Gewicht und die Zusammensetzung der Leber bei Kälbern.

Fünfzehn Kälber wurden nach Körpergewicht in drei Gruppen eingeteilt, in jeder Gruppe fünf. Ein Kalb bei dem man beim Schlach-

ten eine eitrige Gonitis fäststellte, wurde in das Versuchsmaterial nicht aufgenommen. Nach dem Transport wurde die Kontrollgruppe unmittelbar, und die zwei übrigen Gruppen 20 Stunden später, geschlachtet. Eine dieser Gruppen bekam während der genannten Zeit weder Futter noch Wasser. Den Tieren der letzten Gruppe wurde insgesamt zweimal 250 g Glukose in 5%-igen Lösung gegeben. Das Schlacht- und Lebergewicht wurde registriert. In der Leber wurde Wasser, Glykogen, Fett und Rohprotein bestimmt.

Das Lebergewicht var signifikant lägre bei den Kälbern in der Hungergruppe als bei den Kontrolltieren. Der Unterschied im Gewicht var grösstenteils durch die verminderte Wassermenge bedingt, ungeachtet dass der Wassergehalt bei hungernden Kälbern grösser var als bei den Kontrolltieren. Das Hungern verursachte fast vollständiges Verschwinden des Glykogens aus der Leber und im ganzen gesehen auch eine Verminderung der Rohproteinmenge, dagegen eine Erhöhung des Rohproteingehaltes. Die Fettmenge der Leber var erhöht und durch eine leichte, diffuse, feintröpfige Verfettung der Leberzellen bedingt. Die mit der Glukose gefütterte Gruppe hatte beinahe in jeder Hinsicht eine Zwischenstellung zwischen der hungernden und der Kontrollgruppe eingenommen. Anschliessend an die entsprechende Literatur wurde die lebensmittelhygienische und die klinische Bedeutung der Resultate diskutiert.

SAMMANFATTNING

Effekten av ett dygns svält och av glykostillförsel på leverns vikt och sammansättning hos kalvar.

Femton kalvar delades efter kroppsvikten i tre grupper om fem i varje. En av kalvarna befanns efter slakten ha lidit av en purulent gonit, varför den uteslöts ur materialet. En grupp, kontrollgruppen, slaktades omedelbart efter transporten, de två andra 20 timmar senare. Den ena av dessa grupper fick varken vatten eller foder under denna tid, medan den andra tillfördes totalt 250 g glykos, givet som 5-procentig lösning vid två tillfällen. Slaktvikten och levervikten registrerades och leverns sammansättning bestämdes med avseende på vatten, glykogen, fett och råprotein.

Leverns vikt var signifikant lägre hos kalvarna i svältgruppen än hos kontrolldjuren. Viktdifferensen var till största delen betingad av en minskad vattenmängd, trots att vattenhalten i leverna från svältkalvarna var högre än hos kontrolldjuren. Svälten medförde ett nästan totalt försvinnande av leverglykogenet och även totalt sett en minskning av råproteinmängden men en ökning av råproteinhalten. Fettmängden i levern var ökad, vilket betingades av en lindrig, diffus, findroppig levercellsförfettning. Den glykosutfödrade gruppen intog en mellanställning mellan svält- och kontrollgrupp i nästan alla avseenden. Resultatens livsmedelshygieniska och kliniska betydelse diskuteras i anslutning till relevant litteratur.

(Received January 20, 1966).