Onderstepoort Journal of Veterinary Research,
Volume 25, *Number* 1, *January,* 1951.
The Government Printer, Pretoria

Studies on the Alimentary Tract of the Merino Sheep in South Africa. XVIII.—The Effect of Fasting on the Activity of the Ruminal Flora of Sheep and Cattle.

By J. I. QUIN, Section Physiology, Onderstepoort; *W. OYAERT, Veterinary College, University of Ghent, Belgium; and R. CLARK, Section Physiology, Onderstepoort.

Introduction.

Despite the incompleteness of our knowledge regarding the precise rôle of the various types of micro-organisms in the forestomach of ruminants, enough information is nevertheless available to convince us of their very important significance both in the disintegration of fibrous plant materials and in the synthesis of proteins and vitamins.

While these processes are primarily intended to meet with the nutritional requirements of such a composite microflora, the resulting products of metabolism are in turn made available for utilization by the host animal itself.

In a comprehensive review which appeared recently (Nutr. Abstr. and Rev., 1947) this subject was carefully considered in a series of five articles compiled by various authors.

In view of the great importance of micro-organisms in the digestion and general metabolism of ruminants, unlike that of other animals, it is essential to know how this flora is affected by changes in the diet and especially by periodic fasting and prolonged starvation and also how the composition and activity of this mixed flora is to be correlated with a loss in appetite and subsequent digestive disturbances.

In this report various experiments are recorded in which an attempt was made to throw further light on this very complex problem.

Experimental Animals.

Twelve sheep with permanent ruminal fistulae were employed. Six were kept on a ration of grass hay while the other six received lucerne hay, both rations being given *ad lib.* but without any supplements. In addition three cattle also with permanent fistulae were used. These were fed first on grass hay together with lucerne hay and maize and later on lucerne hay alone. The consumption of both food and water was measured daily. During the fasting periods water was allowed *ad lib.*

*This work was done while W. Oyaert was on a study visit to Onderstepoort.

Received for publication on 7th February, 1949.—Editor.

The Effect of Fasting on the Consistence of the Ruminal Contents.

In order to demonstrate the effect of fasting on the consistence of the ruminal contents, samples of ingesta were taken from the fistulae at intervals during fasting. The material was centrifuged at 6,000 r.p.m. for one minute in graduated 10 c.c. tubes and the relative proportions of solid material and fluid recorded. A typical result of several such trials is given in Table 1.

Table 1.

The Effect of Fasting on the Consistence of the Ruminal Contents of a Sheep on a Ration of Lucerne Hay.

Time of Collection.	Fluid Per Cent.	Water Consumption (Litres per 24 hours).
Before Fasting	43	1·7
After 24 hours fasting	58	0·0
After 48 hours fasting	74	0·0
After 72 hours fasting	85	0·0
24 hours after feeding	60	0·2

The ruminal ingesta invariably became progressively more watery during fasting despite the fact that the water consumption was always greatly reduced. This was also found to be the case in cattle.

The Effect of Fasting on the Fermentation of Glucose by Ruminal Ingesta.

Quin (1943) showed that fasting caused a marked decrease in the ability of the ruminal flora to ferment glucose. This was confirmed in the present experiments.

The Effect of Fasting on the Rate of Cellulose Digestion.

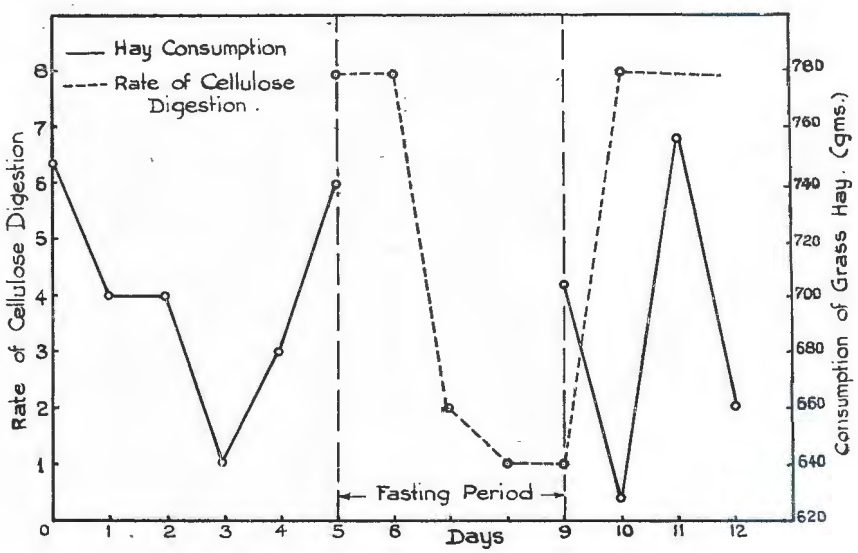
During the above experiments samples of ruminal ingesta were also tested for their ability to digest cellulose *in vitro*. For this purpose the cotton thread technique as described by Hoflund, Quin and Clark (1948) was used.

In the case of sheep on grass hay it was found that the threads were normally completely digested in 36 to 48 hours. The rate of cellulose digestion was not affected after 24 hours fasting but it was markedly retarded in samples taken 48 hours or longer after the food had been removed. In such samples there was no change in the threads after 96 hours incubation. The rate of cellulose digestion was, however, restored to normal within 24 hours after feeding had recommenced. It was also noted that, when the grass hay ration was again offered after a period of fasting, the sheep immediately resumed feeding at the pre-fasting level.

Similar observations were also carried out on a group of sheep fed on a ration of lucerne hay only. It was found that fasting for 48 hours had the same effect of depressing cellulose digestion but that in this case recovery was delayed for two to three days after feeding was resumed. Moreover the appetite was gradually recovered in contrast to the immediate resumption of normal feeding as shown by the sheep on grass hay. These results are shown graphically in Graphs 1 and 2.

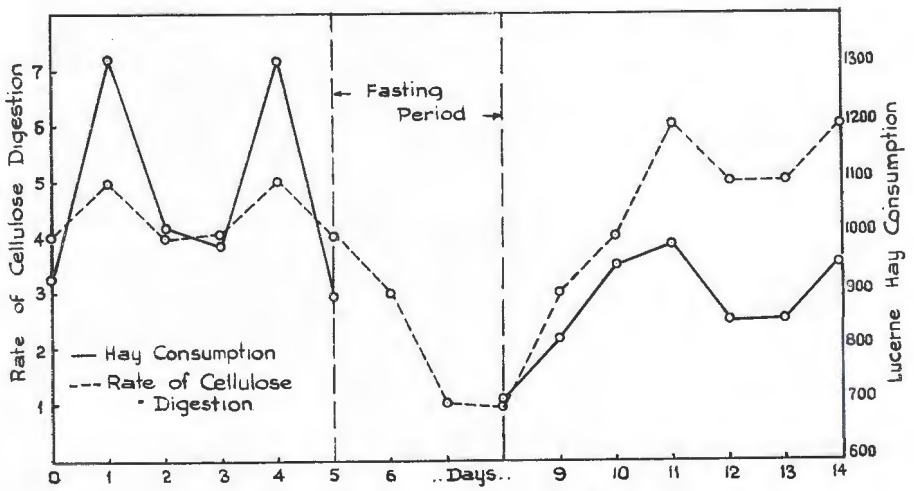
GRAPH 1.

The Effect of Fasting on the Rate of Cellulose Digestion and Food Consumption in Sheep on a Ration of Grass Hay.



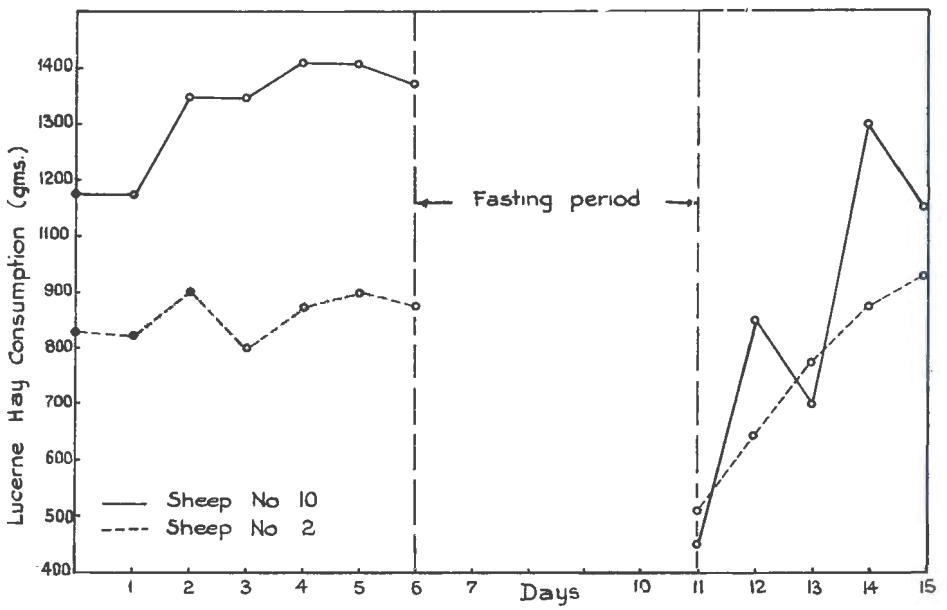
GRAPH 2.

The Effect of Fasting on the Rate of Cellulose Digestion and Food Consumption in Sheep on a Ration of Lucerne Hay.



The very close correlation between the consumption of hay and the rate of cellulose digestion as determined *in vitro* by the cotton thread technique of Hoflund *et al* (1948) is again demonstrated in the above graphs. This is further proof that this test accurately reflects the cellulose splitting powers of the ruminal flora at the time of sampling. In contrast to the rapid recovery of appetite on a diet of grass hay the gradual return of appetite after fasting has been repeatedly noted in sheep on a ration of lucerne hay. Two further examples are shown in Graph 3.

GRAPH 3.

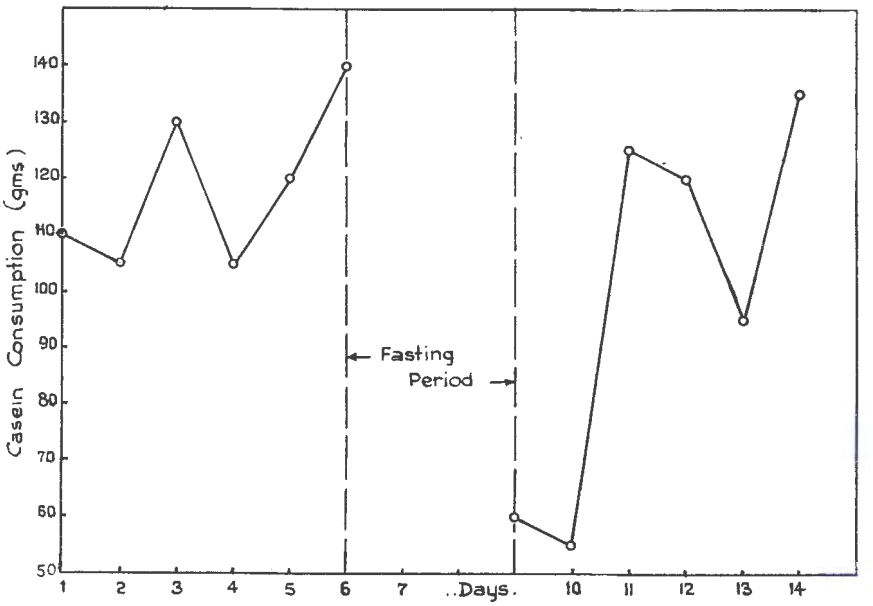


Hoflund *et al* (1948) showed that the sudden introduction of as little as 50 gm. of casein per day into the diet of a sheep adapted to a low protein intake (veld hay only) caused an immediate and marked drop in appetite. The consumption, however, gradually returned to normal as the ruminal flora became adapted to the higher protein level. This suppression of consumption could be avoided by the simultaneous administration of readily available carbohydrate in the form of molasses.

As the lucerne hay used contained 15 per cent. protein and was comparatively low in readily available carbohydrate (starch and sugar), the inappetence for this diet after starvation was considered to be due to the relative preponderance of protein. In order to test this theory the following experiment was carried out. Two sheep were placed on a ration of poor quality grass hay with a protein content of 2 per cent. This was supplemented by 150 gm. of casein per day which was offered in a separate container. The daily consumption of both grass hay and casein was recorded. After the animals had become adapted to the diet all food was removed for 48 hours after which feeding was recommenced as before. The effect of the fasting on the consumption of casein is shown in Graph 4 (average for the two sheep).

GRAPH 4.

The Effect of Starvation on the Daily Consumption of Casein



Immediately after starvation the consumption of hay was resumed at the pre-fasting level as indicated in Graph 1 but, as will be seen from Graph 4, the intake of casein was very low for the first two days, approximately 50 gm. daily as against 120 gm. normally. This would indicate that sheep automatically regulate the intake of protein to correspond with the ability of the ruminal flora to metabolise it.

THE EFFECT OF STARVATION ON THE FIXED AND FREE CELLULOSE SPLITTING BACTERIA.

The cellulose splitting organisms of the rumen may be divided into two types, the one being free in the fluid and the other adherent or embedded in the plant particles. In the present tests on the *in vitro* digestion of cellulose by ruminal ingesta during starvation the following facts were noticed:—

1. In ingesta taken before starvation digestion of the cotton thread in the test tube occurred throughout the submerged length.
2. In samples taken during starvation digestion took place only in the bottom of the tubes, i.e. the sediment.

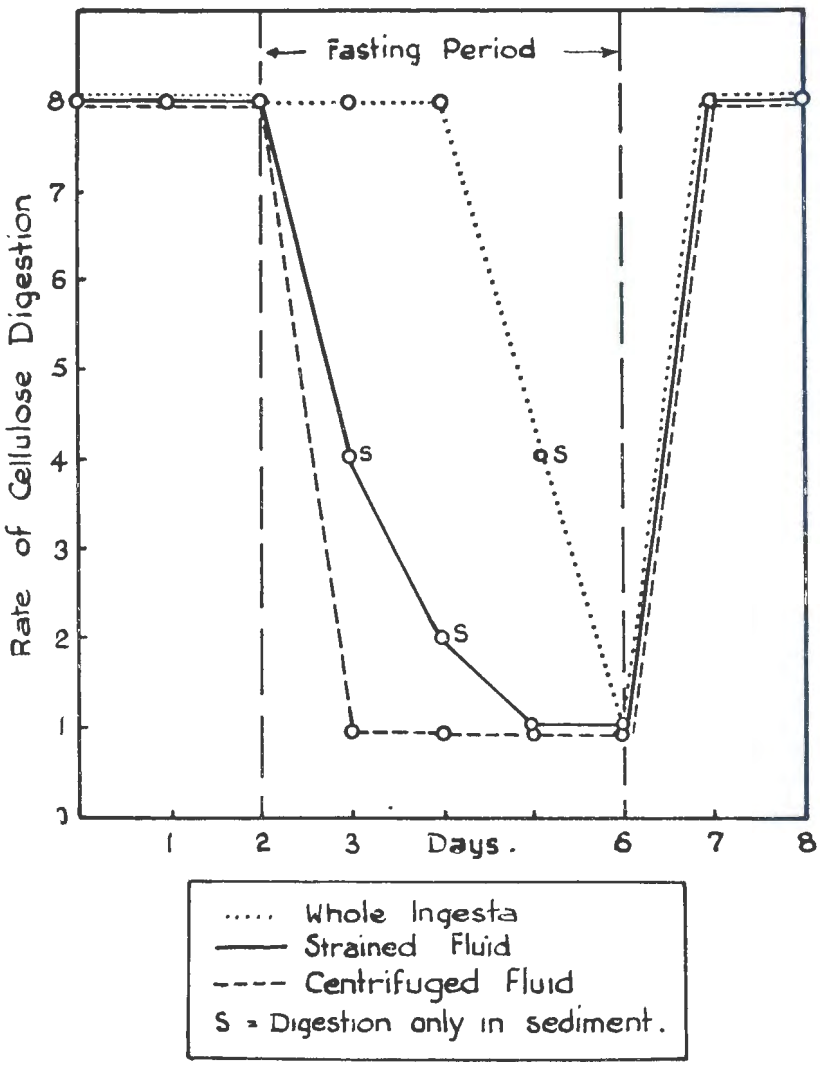
This observation indicated that starvation not only retarded cellulose digestion but that it affected the free bacteria before the fixed organisms. In order to test this further the digestion of cotton threads was tried in the following fractions of ingesta, viz.:—

1. The whole ingesta as removed.
2. The fluid portion after straining through butter-muslin.
3. The supernatant portion after centrifuging this fluid at 6,000 r.p.m. for one minute.

These observations were carried out before, during and after starvation respectively. The results are given in Graph 5.

GRAPH 5.

The Effect of Starvation on the Free and Fixed Cellulose Splitting Bacteria.



As will be noted from the graph the cellulose splitting capacities of all three fractions were equal prior to starvation. The fluid portion of the ingesta showed a marked drop in activity within 24 hours after the food had been removed whereas the whole ingesta showed no change for a period of 48 hours starvation.

This experiment shows that during starvation the free cellulose splitting organisms disappear probably both by passage down the tract as well as by fixation to the rapidly diminishing number of food particles.

The above experiment was also repeated using strips of fine cellophane in place of the cotton thread. The digestion of the cellophane could be judged by direct observation and after staining with chlor-zinc-iodine. The results were the same as with cotton thread.

The Cause of the Retardation of Cellulose Digestion During Starvation.

The loss in power to digest cellulose shown by ruminal ingesta during starvation may be due to either (1) disappearance of cellulose splitting organisms or (2) inability of the organisms present to attack cellulose in the absence of other essential nutrients. It was found that the addition of boiled normal ruminal fluid to the ingesta of a starved sheep did not promote cellulose digestion but that this was attained by adding small amounts of chopped hay even to boiled ingesta from starved sheep. The sudden recovery in cellulose digestion after the resumption of feeding would therefore appear to be due to bacterial contamination of the hay.

Results with Cattle.

Similar comparative experiments were carried out on the fistula cattle and the results with regard to cellulose digestion were found to be very similar. It wat noted, however, that cattle returned to their normal consumption of lucerne hay immediately after starvation. This may indicate that cattle are less sensitive than sheep to changes in inter-ruminal conditions and may partially explain the fact that they are so much more prone to digestive disturbances.

Discussion.

The above experiments show that starvation for over 48 hours markedly affects the composition and activity of the ruminal flora and that these factors must be restored before normal digestion can again take place. In cases of sick animals where anorexia has been present for any considerable time, care should be taken with the diet on the recovery of appetite. Under such conditions it would appear that excessive protein should be avoided until the ruminal flora has been restored.

From the observations on the pseudo-yeasts and cellulose splitters it would appear further that the functionally important types of ruminal organisms are not of a permanent character but require constant replenishment from natural contamination with the food.

Summary.

1. The effects of starvation on the consistence of the ruminal ingesta and the activity of the ruminal flora have been studied.

2. The ruminal contents were found to become progressively more watery during starvation although there was still a large volume present even after 96 hours without food.

3. Both the fermentation of sugar and the digestion of cellulose were markedly depressed after 48 hours starvation.

4. On a low protein diet of grass hay the appetite returned to normal immediately after starvation but on a higher protein diet of lucerne hay the consumption after starvation was low and only returned to normal after 3 to 5 days. There was evidence to show that the sheep regulated their protein intake in accordance with the adaptation of the ruminal flora. This did not appear to apply to the same extent to cattle.

REFERENCES.

HOFLUND, S., QUIN, J. I. AND CLARK, R. (1948). Studies on the Alimentary Tract of Merino Sheep in South Africa. XV. The influence of different factors on the rate of cellulose digestion (*a*) in the rumen (*b*) in ruminal ingesta as studied in vitro. *Onderstepoort Jnl.* Vol. 23, Nos. 1 and 2, pp. 395-409.

QUIN, J. I. (1943). Studies on the alimentary tract of merino sheep in South Africa. VII. Fermentation in the forestomachs of Sheep. *Onderstepoort Jnl.* Vol. 18, Nos. 1 and 2, pp. 91-112.

SMITH, J. A. B. AND BAKER, F. (1944). The utilization of urea in the bovine rumen. 4. The isolation of the synthesized material and the correlation between protein synthesis and microbiological activities. *Biochem. Jnl.* Vol. 38, pp. 496-505.

Various authors (1947). The role of the microflora of the alimentary tract of herbivora with special reference to ruminants. *Nutr Abstr. and Rev.* Vol. 17, No. 1, pp. 1-37 .