A Separation Mechanism and Fluid Flow in the Large Intestine of the Equine

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Summary

A ration of mainly straw and hay was given to donkeys and ponies with polyethylene glycol as a reference substance. At slaughter the intestine was divided into segments. From the amount and composition of the contents of these the flow and resorption of fluid along the intestine were calculated.

It could also be shown that retention of fluid and small particles occurred at the boundary between dorsal colon and distal colon. This retention results in a considerable retention of microorganisms (and presumably of bacterial activity) in the dorsal colon, and an unusually low concentration of nitrogen in the faecal matter. Most of the net fluid resorption occurred in the caecum and in the dorsal colon.

Separationsmechanismen und Flüssigkeitsbewegungen im Dickdarm des Pferdes

Eine Ration, die hauptsächlich aus Stroh und Heu bestand, wurde mit Polyethylenglycol als Marker an Esel und Ponys verfüttert. Nach der Schlachtung erfolgte eine Unterteilung des Darms in Segmente. Aufgrund von Menge und Zusammensetzung des Darminhalts in diesen Abschnitten wurde der Chymusfluß und die Flüssigkeitsresorption im Darmkanal ermittelt.

Weiterhin konnte gezeigt werden, daß es am Übergang vom dorsalen zum distalen Colon zu einer Retention von Flüssigkeit und feinen Partikeln kommt. Diese Retention ergibt sich aus einem erheblichen Zurückhalten von Mikroorganismen (und vermutlich mikrobieller Aktivität) im dorsalen Colon und ungewöhnlich niedrigen Stickstoffkonzentrationen im Kot. Der größte Anteil der Nettoresorption von Flüssigkeit findet im Caecum und dorsalen Colon statt.

Introduction

In the Scandinavian lemming the faecal pellets are almost free of microorganisms, whereas the contents of the first part of the colon largely consists of bacteria. From this observation and a study of the anatomically very complex colon of this species *Sperber* (1968) concluded that a colonic spearation mechanism (CSM) existed in this animal, separating bacteria and returning them to the caecum, to the benefit of the lemmings.

A somewhat similar mechanism was studied by *Björnhag* in the rabbit (1972). Later CSMs have been found in many herbivores. The space does not permit a review of the various investigations in this field, which has been reviewed repeatedly e. g. by *Björnhag* (1989), *Stevens* (1988) and *Sperber* (1985).

Most of the animals which have been shown to possess a CSM are small, and there is little doubt that they are especially valuable in small species.

It appeared to us that large species could also benefit from CSM, and observation with regard to the consistency of the faecal balls of horses when eating largely straw and hay led us to investigate the possible existence of a CSM in equines.

Materials and Methods

Eight. donkeys (body weight 80 – 130 kg) and two ponies (body weight 100 kg) were used. The two first donkeys were used to develop the methods. They gave quite similar results as the following but are not included in the statistical treatments.

All animals were given oat straw ad lib. and small amounts of hay and crushed oats (0.5 respectively 0.3 – 0.5 kg per day).

All animals got polyethylene glycol (PEG, MW 4000) as a water soluble, non absorbable marker during the last week before they were killed. The marker was given on moistened crushed oates every six hours. In the six last of our animals the marker was given every three hours during the last day, and at the end once per hour during the last three hours of the experiment.

The animals were then anaesthetized at different times of the day and night by pentobarbital intravenously. They were secured in the standing position and the different parts of the intestine were tied off and the animals were exsanguinated.

The marker concentrations are given as percentages of the mean values measured in the contents of the distal colon of the same animal.

The following analyses were made: PEG, nitrogen, acid detergent fibre (ADF), dry matter (DM). Particles of the digesta were fractionated according to size by wet sieve analysis. Particles which passed through 0.1 mm mesh size are called small particles.

The fraction of fluid spontaneously separating from the digesta of the large intestine was estimated. The digesta was placed on a coarse sieve (mesh size 5 mm), and the amount of fluid (with small particles) running through was measured. The concentrations of DM, nitrogen and PEG were measured in the fluid.

The significances of the found differences were tested with the Students t-test.

Results and Discussion

In general breakdown and absorption of food components proceeds all along the intestine. For this reason the ratio of the dry matter concentration and the concentration of a

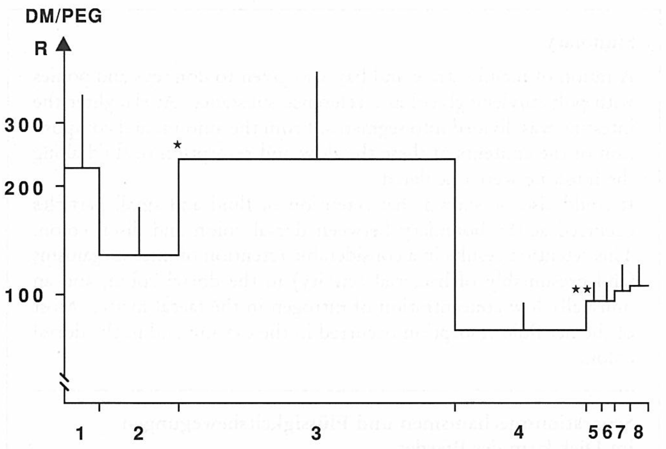

Fig 1. The DM/PEG ratio in intestinal contents of six donkeys and two ponies.
R = relative to the mean ratio in distal colon. 1. small intestine, 2. caecum, 3. ventral colon, 4. dorsal colon, 5. – 8. distal colon. * = $P < 0.05$, ** = $P < 0.01$. The bars represent standard deviations.

nondigestible reference substance will in general decrease along the intestine. Thus any statistically significant rise in this ratio merits attention, and if possible explanation. We may use fig. 1 for this purpose, assuming PEG to be such a reference substance. In the curve shown there are two points at which a marked rise of the ratio DM/PEG occurs. The first is at the boundary between the caecum and the ventral colon. The rise is barely significant ($P < 0.05$) but may nevertheless be of interest. The rise in DM/PEG ration indicates that dry matter passes more rapidly out of the caecum than the fluid. The function of the anatomically complicated boundary between caecum and colon has been the object of several investigations, but it does not appear that anyone has tried to quantify the outflow of dry matter and fluid through the orifice in question.

The second significant rise of the DM/PEG ratio ($P < 0.01$) occurs at the beginning of the distal colon (fig. 1). With regard to the statistical significance of observed changes in the composition of the colonic contents the boundary between dorsal colon and distal colon is the most marked of all the boundaries found in the equine digestive tract. Significant ($P < 0.001$) changes occur with regard to N/DM (fig. 2), ADF/DM, particles smaller than 0.1 mm/DM, and proportion of the fluid fraction (table 1). No species differences between donkeys and ponies were found in the analyses.

In the distal colon the composition of the dry matter with regard to nitrogen, PEG, proportion of small particles and ADF are remarkably constant along the organ (table 1). In no case the contents of the distal colon gave off fluid when placed on a sieve. The dry matter concentration on the other hand shows a steady and relatively strong increase along the distal colon from 16 to 23 % (table 1). These facts together convincingly show that of the parameters measured in this investigation only the water content is significantly changed when colonic contents pass along the distal colon.

The considerable drop from dorsal colon to distal colon with regard to concentration of small particles, of nitrogen and of PEG/DM is quite remarkable when compared to the insignificant very small change in these components along the distal colon. It is thus very improbable that the change could be ascribed to destruction or resorption of them in transverse colon or the very first part of the distal colon. This is especially the case with regard to PEG/DM. The change in ratio PEG/DM must in the main be due to either selective retention of PEG in the dorsal colon or preferential transfer of dry matter into the distal colon. Retention of PEG of course means preferential retention of fluid, which would also explain preferential retention of small particles. The small particles clearly to some extent consist of microorganisms, and the free-flowing fluid is high in N/DM (average 33 mg/g in dorsal colon).

The retention of fluid (or preferential transfer of dry matter) can thus explain the marked drop in nitrogen concentration at the proximal boundary of the distal colon. Similarly the increase in acid detergent fibre may also be explained by retention of fluid and preferential transfer of large particles, which to a considerable extent must be remnants of the most indigestible parts of the food.

As to the mechanism responsible for the observed partial separation and retention of the more fluid part of the colo-

Tab. 1: Concentrations of dry matter (DM), of acid detergent fibre (ADF) in DM, of small particles (SP, < 0,1 mm) in DM and of free flowing fluid (FFF) in contents of the digestive tract of six donkeys and two ponies (DM, SP/DM, FFF), or in four donkeys and two ponies (ADF/DM).

	DM		ADF/DM		SP/DM		FFF	
	%	SD	%	SD	%	SD	%	SD
Stomach	23.1	3.7	34.4	3.5	39.9	6.4	–	–
Caecum	8.8	1.2	46.1	5.3	32.8	1.7	35.0	11.6
Ventral colon	13.4	1.7	46.4	2.7	33.2	6.4	10.7	3.7
Dorsal colon	12.6	1.4	40.6	2.8	41.6	5.2	21.1	4.0
Distal colon 1	16.6	2.1	46.2	0.9	28.4	5.2	0.0	0.0
Distal colon 2	19.2	3.0	47.6	0.8	29.4	10.4	0.0	0.0
Distal colon 3	21.2	3.0	48.1	2.2	26.3	10.3	0.0	0.0
Distal colon 4	22.9	2.8	47.6	2.5	26.6	4.4	0.0	0.0

SD = standard deviation

nic contents two possibilities appear possible and likely. One is the combination of retrograde peristalsis and squeezing of each portion of the colonic contents as it passes through the transverse colon and passage of fluid back into the dorsal colon.
Another possibility is that the contents of the right dorsal colon are slightly separated in a lower part containing a higher proportion free fluid and upper part containing less fluid and instead containing a higher proportion of gas. As the upper part will undoubtedly preferentially enter the transverse colon, the observed effect is explicable by this mechanism also or rather by a combination of both possibilities.
The separation mechanism clearly leads to the retention in the dorsal colon of water with dissolved substances, small particles and nitrogenous substances. The retention of water and solutes will lead to resorption of these in the dorsal colon and not in the distal colon. This result is probably of little importance.
The small particles retained may be considered as consisting of two classes. One is particles which are mainly a result of mechanical subdivision of larger food particles during the passage through the digestive canal. These particles have been partly digested during this passage, and there is no evidence to show that they at the end of this passage are more digestible than the larger particles. The second class of small particles consists of microorganisms. These are obviously nutritionally quite important. They are highly digestible and have high content of protein. It must be this class which is mainly responsible for the high nitrogen concentration of dry matter in the dorsal colon which is about twice as high as in the distal colon and considerably higher than in the ventral colon (both these changes are significant, $P < 0.001$). Clearly the retention to a considerable extent of the microorganisms in the dorsal colon is largely responsible for the exceptionally low nitrogen concentration of the faecal matter in our experimental animals.
In addition to this nutritionally important effect the high microorganism concentration in the corsal colon must result in a higher rate of fermentation in the dorsal colon than would be the case without the separation mechanism. These advantages are obviously of special importance when the animal is obliged to subsist on food of low digestibility and/or low nitrogen content. Such is the case when the food consists to a large extent on mature grasses, i. e. during cold or dry seasons. Such food is obviously at the same time very suitable for the functioning of the separation mechanism.
The conclusion appears unavoidable that at the boundary of dorsal colon and distal colon there is some mechanism preferentially retaining fluid compared to coarse particulate matter.
This existence of a colonic separation mechanism is of special interest as it occurs without any connection with the caecum, and is apparently not linked to caecotrophy, either or both of which is the case in all previously described colonic separation mechanisms.
The increased utilization of nitrogen may also be of special value for the equines who have unusually long gestation periods.
Clearly the separation mechanism allows a high nitrogen concentration (= high microbial activity) in the dorsal colon without a high nitrogen loss in the faeces.

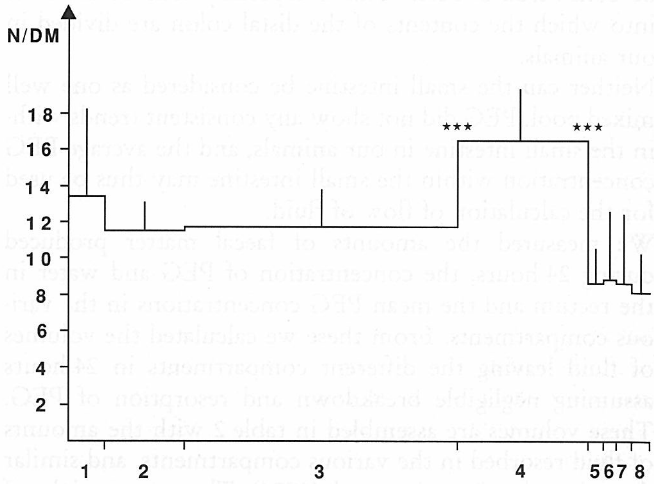

Fig. 2 Concentration of nitrogen in dry matter (mg N/g DM) in intestinal contents of six donkeys and two ponies. 1. small intestine, 2. caecum, 3. ventral colon, 4. dorsal colon, 5. – 8. distal colon. *** = $P < 0.001$. The bars represent standard deviations.

Fluid flow and resorption in the colon
The six last of our animals were given food and PEG in such a manner that a steady state could be expected at least

Tab. 2: Calculated flow of water in four donkeys and two ponies. Average body weight 99 kg. Volumes in litre/24 h.

	This study		*Argenzio* et *al.*	
	outflow	net change	outflow	net change
Stomach	8.1			
Small intestine	25.4	+17.3	19.4	
Caecum	13.9	−11.5	6.8	−12.6
Ventral colon	12.2	− 1.7	2.5	− 4.3
Dorsal colon	6.7	− 5.5	3.3	+ 0.8
Distal colon	3.8	− 2.9	1.5	− 1.8

in the small intestine and more distal parts of the intestine. The results appear to support this expectation. Such a state allows the calculation of the flows of water through the different segments of the digestive canal provided may be considered at least approximately separate, mixed pools. Our data, in agreement with those of *Argenzio* et *al.* (1974), support this assumption with regard to the caecum, the ventral colon and the dorsal colon. In our data (contrary to these authors) the distal colon cannot possibly be considered as one mixed pool, but small segments of this part may well be considered as such. This is especially true of the balls into which the contents of the distal colon are divided in our animals.

Neither can the small intestine be considered as one well mixed pool. PEG did not show any consistent trends within the small intestine in our animals, and the average PEG concentration within the small intestine may thus be used for the calculation of flow of fluid.

We measured the amounts of faecal matter produced during 24 hours, the concentration of PEG and water in the rectum and the mean PEG concentrations in the various compartments. From these we calculated the volumes of fluid leaving the different compartments in 24 hours assuming negligible breakdown and resorption of PEG. These volumes are assembled in table 2 with the amounts of fluid resorbed in the various compartments, and similar data given by *Argenizo* et *al.* (1974). The mean weight of their ponies was 160 kg, but they did not consume significantly more food than our animals weighing on average 99 kg. *Muuss* et *al.* (1982) found that the flow from the small intestine was 9.9 l/kg dry matter consumed in highly digestible pellets, but the flow was 12.0 - 14.2 l when a mixture of pellets and straw or exclusively hay or straw was given. Clearly our figures for the flow from the small intestine are in fair agreement with those of other authors. The greatest discrepancy between our findings and those of *Argenzio* et *al.* (1974) is with regard to the dorsal colon. They report a net influx of 0.8 l/24 hours and we find a resorption of 5.5 l, which is more than in any other part of the colon. At least part of the discrepancy may be ascribed to the fact that they gave food every twelfth hour during only one hour, whereas our animals had access to food almost continuously.

Retrograde flow of fluid from the distal to the dorsal colon has no appreciable influence on this mode of calculation as it occurs at the very boundary between the segments in question.

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