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ABSTRACT

In this experiment certain physiological properties of the digestive tract were examined and compared in young rabbits suckled either once (SS) or twice (DD) daily up to the age of 35 days. The quantity of the gastric content was found to rise with advancing age in both groups (from 36 to 70 g and from 37.5 to 79 g). In both groups the pH of the stomach was higher during the suckling period (4.5-5.3) than subsequent to weaning (1.6-2.4). The quantity of the caecal content also increased with age (from 4 or 8 g), on the 37th day significantly higher quantities of caecal content were recorded in DD rabbits (50.5 g compared with 35 g). In these rabbits the pH of the caecal content decreased more slowly from an initial high value (6.0 and 6,5 in SS and DD rabbits, respectively), and by the 37th day had settled at a low value (5.7-5.8) in both groups. The dry matter content of the content of the caecum decreased from 27 % to 19-21 % in both groups. Total volatile fatty acid (tVFA) and acetic acid (C2) concentration, which amounted to 66-88 % of tVFA, rose; in SS rabbits they were at higher levels by the 30th day (53.6 and 42.2 mmol/L, respectively), and remained at an increased level until the 44th day ($P<0.05$). The ratio of C3:C4 was high on the 23rd day (2.5 and 2.4), but had decreased by the 30th day (0.5 in SS and 1.7 in DD) and, further, by the 40th day (0.2). In SS rabbits coliform count proved significantly lower (10^5) on the 23rd day than in DD rabbits (10^6). By the 37th day this count had decreased in both groups and subsequently remained at a low level ($<10^4$). By the 23rd day Bacteroides were present in large quantities (10^8) in the caecum and showed no change with advancing age.

INTRODUCTION

Young rabbits reared by a traditional method suckle only once a day (Lebas, 1970; Szendrő et al., 1993). Up to the age of three weeks young rabbits are nourished exclusively by milk (Lebas et al., 1986; Kustos et al., 1996), after which they gradually adapt to solid feed. Young rabbits are weaned at 28 days at the earliest in practice, but most commonly between 35 and 42 days of age (Cheeke, 1987).

Experiments performed with meat type rabbits (McNitt and Moody, 1988; Szendrő et al., 1988; Gyarmati et al., 1999) have provided clear-cut evidence that young rabbits do show an inclination to suckle twice daily, and thus have access to 80 to 85 % greater quantities of nutrients than those suckled only once a day. The weight advantage attained in this way is maintained during the fattening period. On the basis of a number of findings relating to mortality (Szendrő et al., 1998) it can be assumed that problems from the aspect of digestion physiology will be encountered in the adaptation to solid feed after weaning in young rabbits which have previously suckled twice a day.

The aim of this experiment was to examine and compare certain physiological properties of the digestive tract in young rabbits suckled either once or twice daily up to the age of 35 days.

MATERIALS AND METHODS

The Pannon White rabbits used in these investigations were either reared by a traditional method, i.e. suckled once a day from birth until weaning at the age of 35 days and allowed to adapt to solid feed at a normal pace (SS), or suckled twice a day by two mothers from birth until weaning at the age of 35 days (DD). The additional daily suckling was carried out by an additional doe which had produced a litter at the same time but whose progeny had been removed. One doe was put into the nest box at 8 a.m. every day. For the second nursing the other doe was put in at 8 p.m. Suckling twice a day was maintained until weaning, i.e. until the age of 35 days.

The young rabbits were fed an unmedicated rabbit feed (CP: 16.8%, CFat: 2.9%, CF: 14.1%, 10.3 DE MJ/kg), supplemented with barley and hay. The various feedstuffs provided were available *ad libitum*, and the rabbits also had free access to drinking water from weight-valve self-drinkers.

On the 23rd, 30th, 37th and 44th days subsequent to birth 4 animals from each group were slaughtered. After the digestive tract had been removed and the stomach, small intestine and caecum had been separated, the following parameters were determined:

The quantity of the gastric and caecal content was measured. The pH value of the fresh gastric, intestinal and caecal content was determined by a manual automatic pH meter (OP-110, Radelkis, Hungary).

The dry matter content of the chyme samples was determined according to Hungarian Standard MSZ 6830-77.

Volatile fatty acid composition of the caecal content (acetic, propionic, butyric, isobutyric, valeric, isovaleric and capronic acid) was conducted using gas chromatographic method. 1g sample was diluted with 10 ml deionised water, then mixed with 85% phosphoric acid. The samples were centrifuged for 10 minutes. The supernatant was collected, frozen for 24 hours and then filtered. 1 ml diethilether was given to 1050 µl of the supernatant, and then injected into the Chrompack CP 9000 gas chromatograph. FFAP 30m x 0.32 mm capillary column and flame ionisation detector were used.

For bacterial assays, serial dilutions were made from 1 g aliquots of chyme. The counts of Bacteroides, coliforms, lactobacilli and streptococci were determined as follows: the Bacteriodes spp. were cultured on Schaedler agar, supplemented with esculine, neomycine and Fe-ammonium-citrate, in anaerobic thermostat at 37°C for 96 hours. Lactobacilli were cultured on MRS medium, in anaerobic thermostat at 37°C for 48 hours. Streptococci were cultured on Edward's medium, in aerobic thermostat at 37°C for 48 hours. The total count of the coliforms was determined on Drigalsky's medium, in aerobic thermostat, at 37°C for 24 hours. After their respective incubation periods, colonies were visually counted.

Statistical analyses were performed using the SPSS (1996) programme package. Differences between the groups and between the dates of examination were determined by paired t-test or analysis of variance.

RESULTS AND DISCUSSION

Quantity and pH of the gastric and caecal content (Table 1)

The quantity of the gastric content was found to rise with advancing age in both groups. The slight degree of difference between the two groups did not prove significant at any point. It seems that double suckling or earlier solid feed intake did not have significant effect on the quantity of the gastric content.

In both groups the pH of the stomach was higher during the suckling period than subsequent to weaning; in the rabbits suckled twice a day gastric pH was observed to fall more slowly, not until the 44th day reaching the value characteristic of fully developed rabbits.

The quantity of the caecal content also increased with age. It was presumably in consequence of the more rapid growth of the organs which occurred due to faster growth in the rabbits suckled twice daily that on the 37th day significantly higher quantities of caecal content were recorded in these rabbits, but this difference had narrowed by the 44th day. In the rabbits suckled twice daily the pH of the caecal content decreased more slowly from an initial high value, and by the 37th day had settled at a low value in both groups.

Throughout the experimental period the pH of the chyme sampled from the small intestine varied between 6.8 and 7.1; no significant difference was ascertained either between samplings or between groups.

Table 1. Quantity and pH of the gastric and caecal content

Groups	***Age (days)***							
	23		***30***		***37***		***44***	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
	Weight of the gastric content (g)							
SS	36	5	47	4	53	5	70	6
DD	37.5	4.5	50	5	64	6	79	8
pH of the gastric content								
SS	5.3	0.2	4.6	0.5	1.8*	0.2	1.6	0.3
DD	5.2	0.3	4.5	0.4	2.4*	0.3	1.6	0.2
Weight of the caecal content (g)								
SS	4	1.5	16	3	35*	4	66.5	7
DD	8	2	20	4	50.5*	5	77	8
pH of the caecal content								
SS	6*	0.3	6.1	0.2	5.8	0.3	5.7	0.4
DD	6.5*	0.2	6.3	0.4	5.8	0.4	5.8	0.3

** significant difference between groups (P<0.05)*

Dry matter content and VFA concentration of caecal chyme (Table 2)

The dry matter content of the caecal chyme decreased from 27 % to 19-21 % in both groups with advancing age. Total volatile fatty acid (tVFA) concentration increased; in the rabbits suckled once daily this was at a higher level by the 30th day, and remained at an increased level until the 44th day (P<0.05).

In correspondence with data in the literature (Padilha et al., 1995), the concentration of acetic acid (C2), which amounted to 66-88 % of tVFA, was found to change in parallel with the curve obtained for total volatile fatty acid concentration, though the differences between groups could not be statistically proven.

On the 23rd day, alongside acetic acid, propionic acid (C3) was found to be in the greatest quantity (amounting to 16 or 13 % of tVFA); with advancing age this quantity subsequently decreased, while that of butyric acid (C4) rose. In correspondence with the changes in tVFA observed, higher acetic and butyric acid concentrations were measured by the 30th day in the rabbits suckled once daily, while at the same time propionic acid concentration was found to be at a higher level in the rabbits suckled twice daily.

The ratio of C3:C4 was high on the 23rd day, but had decreased sharply by the 30th day and, further, by the 40th day. The quantity of the minor volatile fatty acids (mVFA), i.e. branched chain fatty acids (isobutyric, isovaleric) and valeric acid was low (1.8 mmol/L) by the

23rd day in the rabbits suckled once daily, and showed no subsequent change. The concentration of these fatty acids in the rabbits suckled twice daily was 4.1 mmol/L on the 23rd day and 4.9 mmol/L on the 30th day, but by the 37th day the value had fallen to 0.8 mmol/L.

The findings of this study show a similar tendency to that of the experimental results obtained by Padilha et al. (1996) with rabbits suckled twice daily up to the age of 42 days, reared exclusively on milk. In comparison with the tVFA concentration of 12.5 mmol/L measured by the above authors, in the present study the caecal content was found to contain substantially higher quantities of volatile fatty acids; the reason for this may have been that the rabbits which had been suckled twice a day also ate solid feed from the 25-28th day. This may provide an explanation for the mass colonisation at an early stage by Bacteroides also observed in the rabbits suckled twice daily (see below). In the same experiment performed by Padilha et al. (1996) the concentration of branched-chain volatile fatty acids and valeric acid remained at high levels throughout; in the present study high concentrations were recorded prior to weaning in the rabbits suckled twice daily, which indicates that more intensive proteolytic fermentation processes were taking place.

Table 2. Dry matter content and VFA concentration of caecal chyme

Groups	***Age (days)***							
	23		***30***		***37***		***44***	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
	dry matter content (%)							
SS	27.3	0.8	21.6	0.8	21.1	0.6	20.7	0.6
DD	27.0	0.6	22.1	0.9	21.0	0.7	18.8	0.5
tVFA content (mmol/L)								
SS	29.4	2.8	50.0*	5.6	54.0*	6.2	70.4*	8.2
DD	26.4	3.4	31.2*	4.1	38.4*	5.2	51.1*	6.1
acetic acid (C2) content (mmol/L)								
SS	21.0	1.8	42.2	5.9	43.0	6.5	60.3	8.2
DD	17.5	1.5	27.2	3.4	30.8	5.2	43.3	3.6
propionic acid (C3) content (mmol/L)								
SS	4.7	1.1	2.0	0.2	1.8	0.1	1.5	0.3
DD	3.4	1.8	3.1	0.1	2.9	0.2	1.6	0.2
butiric acid (C4) content (mmol/L)								
SS	1.9	0.5	4.3	0.7	7.4*	1.0	7.6	1.2
DD	1.4	0.4	1.8	0.4	3.9*	0.1	6.1	0.5
C3/C4 ratio								
SS	2.5	0.3	0.5*	0.2	0.2	0.05	0.2	0.05
DD	2.4	0.3	1.7*	0.3	0.7	0.2	0.3	0.1

** significant difference between groups (P<0.05)*

Composition of the caecal microflora (Table 3)

In conformity with data in the literature (Gouet and Fonty, 1979; Fekete, 1988), the intestinal microflora was found to consist (characteristically of the rabbit, but in contrast with other monogastrics) of simple, mainly non-sporulated, strictly anaerobic, Gram-negative Bacteroides. Total anaerobic germ count proved high (10^{10}-10^{11} bacteria per g chyme) in both groups.

In the group suckled once a day coliform count proved significantly lower (105) on the 23rd and 30th day than in the rabbits suckled twice daily (106). By the 37th day this count had

decreased in both groups and subsequently remained at a low level (<104). Padilha et al. (1996) found that coliform count is primarily dependent on the age of the animal, not on its feeding and nutrition. Prohászka (1980) and Morisse et al. (1985) stated that a relation can be demonstrated between coliform count, pH and tVFA concentration. In the present experiment, in addition to higher coliform count, higher pH and slightly lower tVFA concentration were also recorded on the 23rd day in the group suckled twice daily. However, subsequent to weaning, even despite the lower tVFA content coliform count was found to be at a similarly low level to that in the rabbits suckled once a day.

Streptococci were detected only on the 23rd and 30th day, while lactobacilli could not be detected at all.

By the 23rd day Bacteroides were present in large quantities (108) in the caecum; the numbers of these detected showed no change with advancing age. According to the findings of studies by Gouet and Fonty (1979) these bacteria rapidly become established in the caecum and the colon, as early as the 1st to the 2nd week of life. This can be due to the consumption of the few pellets of hard faeces left in the nest by the mother after suckling as it was observed by Hudson et al. (1996).

Table 3. Data on the composition of the caecal microflora

Groups	***Age (days)***			
	23	***30***	***37***	***44***
	means	means	means	means
	Bacteroides (germ count/g chyme sample)			
SS	$2.8x10^8$	$1.8x10^8$	$3.7x10^8$	$5.6x10^8$
DD	$1.2x10^8$	$6x10^8$	$2.6\ x10^8$	$4.6\ x10^8$
coliforms (germ count/g chyme sample)				
SS	$1.5x10^5$*	$4.3x10^4$*	$2.6x10^3$	$4.3x10^3$
DD	$5.5x10^6$*	$2.9\ x10^6$*	$3\ x10^4$	$4\ x10^3$
Streptococci (germ count/g chyme sample)				
SS	$3x10^5$	$3.5x10^3$	$< 10^3$	$< 10^3$
DD	$1.7\ x10^5$	$2.2\ x10^3$	$< 10^3$	$< 10^3$

** significant difference between groups (P<0.05)*

CONCLUSIONS

Double suckling or earlier food intake had no significant effect on the quantity of the gastric content and on the dry matter content of the caecal chyme. Whereas suckling twice resulted in higher gastric and caecal pH, lower C2, C4 and higher C3 concentration of the caecal chyme. On the basis of these findings it can be ascertained that in the rabbits suckled twice daily these digestion physiology parameters reached the values characteristic of fully developed rabbits more slowly and at a later stage than in other young rabbits reared by the traditional method of once-daily suckling.

The results obtained, compared with further studies, may provide a solution to disorders in the digestion physiology of double-suckled rabbits arising after weaning, and may assist in establishing appropriate preventive measures from the aspects of animal health and hygiene.

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