Composition of Cottontail Rabbit Milk from Stomachs of Young and Directly from Gland[1]

R. R. ANDERSON, K. C. SADLER, M. W. KNAUER, J. P. WIPPLER, and R. T. MARSHALL
University of Missouri, and
Missouri Department of Conservation, and
Missouri Cooperative Wildlife Research Unit
Columbia 65201

ABSTRACT

Milk samples were from stomachs of 27 nursing cottontail rabbits *(Sylvilagus floridanus)* within 5 min after cessation of nursing. Four milk samples were directly from mammary glands by hand milking aided by tranquilizer and oxytocin. Means and standard errors for 27 stomach samples for total solids, fat, protein, lactose, and ash were 33.6 ± .8%, 13.9 ± 1.7%, 14.6 ± .5%, 2.22 ± .05%, and 2.06 ± .07%. Those values of 4 samples directly from the gland were 35.2 ± .4%, 14.4 ± .4%, 15.8 ± .6%, 2.67 ± .26%, and 2.07 ± .06%. Fatty acid compositions were similar for the groups, except linoleic acid was 30.0% of fatty acids from stomach milk and 24.7% in milk obtained directly. Differences between sampling methods in palmitoleate, stearate, and oleate may have been from differences in season of sampling. Minerals were more concentrated in milk obtained directly, except potassium. Differences between milks of domesticated and cottontail rabbits are discussed.

INTRODUCTION

The Eastern cottontail rabbit *(Sylvilagus floridanus)* is an important game animal throughout the eastern half of the United States. In Missouri, this mammal experiences wide fluctuations in numbers. One possible cause is variation in milk quality. However, an immediate problem in measuring milk constituents from this species was collection of samples. Because a precedent was set for the qualitative analysis of milk harvested from the stomachs of the young (8), this method was chosen as an alternative to harvesting milk from mothers' mammary glands.

To verify analyses of milk taken from stomachs of the young, efforts were unrelenting to harvest milk from glands for comparison. Four milk samples were obtained directly from glands of lactating cottontail rabbits by tranquilizing the doe and giving oxytocin to enable manual expression of milk from the nipples. Samples of milk were analyzed for major ingredients, and the two methods of harvest were compared to determine similarities or differences in composition of milk.

MATERIALS AND METHODS

Methods of Harvesting Milk

Removal from stomachs of young. Cottontail rabbits were maintained in a natural grass environment of 40.5 acres enclosed by wire fencing at a location approximately 16 km south of Columbia, Missouri. Nesting locations and behavior patterns were noted. On designated days of lactation, the mother was observed at the nest nursing the young. Within 5 min after she left the nest (approximately 10 min after covering the nest since cottontails nurse for 5 min), one of the young was removed and sacrificed. The stomach was excised rapidly, and the contents were transferred to a glass vial. Milk samples were frozen immediately and stored at −20 C until analyzed. By this procedure, 15 samples were obtained during April, May, and June of 1971. Twelve samples were collected in the same months during 1972.

Removal from mammary gland. Cottontail rabbits maintained as indicated above were trapped in late lactation (days 12 to 15) during July, 1972, and taken to a laboratory building. Each of four lactating does was tranquilized with an intramuscular injection of .2 ml per-

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TABLE 1. Methods in measuring constituents of milk from cottontail rabbits.

Constituent	Method	Reference source
Total solids	Steam evaporation	(1)
Total ash	Muffle furnace	(1)
Total fat	Roese-Gottleib	(1)
Total protein	Micro Kjeldahl	(1)
Lactose	Orcinol reaction	(11, 12)
Fatty acids	Gas chromatography	(5)
Calcium	Atomic absorption	(13)
Magnesium	Atomic absorption	(13)
Phosphorus	Colorimetry	(1)
Potassium	Flame emission	(10)
Sodium	Flame emission	(10)
Chlorine	Titration	(3)

phenazine (5 mg/ml; Trilafon, Schering) and held in an individual cage overnight. After approximately 24 h of separation from the litter, the doe was injected intravenously with 5 IU oxytocin (.25 ml; POP, Armour-Baldwin) to enhance maximum contraction of myoepithelial cells. The teats were pulled immediately between the thumb and forefinger to express several milliliters of milk per gland into a glass vial. Average volume by each method was 21 ml of milk. Samples were frozen and stored until analyzed.

Chemical Analyses

Milk samples were analyzed for total solids, ash, fat, protein, lactose, fatty acids of milk fat, and seven minerals, calcium, phosphorus, magnesium, potassium, chlorine, sodium, and sulfur. Methods and their references are in Table 1. In all cases except fatty acids, 27 observations were on milk samples from stomachs and 4 on samples directly from the mammary gland. In the case of fatty acids, 5 of the 27 samples were chosen at random to compare with 4 samples directly from the gland. Free fatty acids were included in the preparation and esterification technique.

Statistical Analysis

Comparisons of means for each constituent were between the two methods of milk collection using Student's t test (14).

RESULTS

Percentages of total solids, fat, protein, lactose, and ash for 27 samples of rabbit milk from stomach contents and 4 samples directly from the mammary glands are in Table 2. Difference in lactose concentration between the two methods of milk collection was significant ($P<.05$). No differences were found in percent solids, fat, protein, or ash.

TABLE 2. Percentages of primary constituents in milk from cottontail rabbits.

Method	Number of observations	Total solids (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
From stomachs of young	27	33.6 ± .8*	13.9 ± 1.7	14.6 ± .5	2.22 ± .05	2.06 ± .07
Directly from mammary gland	4	35.2 ± .4	14.4 ± .4	15.8 ± .6	2.67 ± .26	2.07 ± .06
t test		N.S.	N.S.	N.S.	P<.05	N.S.

*Mean ± standard error.
N.S. = not significantly different (P>.05).

TABLE 3. Fatty acid analysis of cottontail rabbit milk fat.

Carbon atoms and unsaturations in chain	% (Mean ± SE) Stomach samples	Direct samples	Difference (P<.05)
C_8	9.2 ± 1.3	9.6 ± 2.1	N.S.
C_{10}	13.3 ± 1.8	14.3 ± 1.4	N.S.
C_{12}	5.8 ± .5	3.8 ± .2	N.S.
C_{14}	2.3 ± .2	2.0 ± .1	N.S.
$C_{16:0}$	16.4 ± .6	18.7 ± 1.4	N.S.
$C_{16:1}$	.7 ± .1	1.0 ± .1	*
$C_{18:0}$	2.5 ± .1	3.0 ± .2	*
$C_{18:1}$	10.8 ± .6	12.7 ± .6	*
$C_{18:2}$	30.0 ± 1.3	24.7 ± 1.2	*
$C_{18:3}$	9.6 ± .5	9.8 ± .3	N.S.
$C_{20:1\text{-}5}$	.2 ± .1	.2 ± .1	N.S.
$C_{22:1\text{-}5}$	.2 ± .1	.2 ± .1	N.S.

*Significantly different (P<.05).
N.S. = not significantly different.

Fatty acid composition of the milk fat of five samples from stomachs and four from the gland directly was analyzed for chain lengths of 8 to 22 carbon atoms, lower chains C-4 and C-6 being omitted from the analyses. Results of these analyses are in Table 3. Low and intermediate chain fatty acids including caprylic through palmitic acids were not significantly different between the two methods of collection. However, differences were significant in the higher chain fatty acids including palmitoleic, stearic, oleic, and linoleic acids (P<.05). No differences were in linolenic acid or in the C_{20} and C_{22} length chains of fatty acids.

Major minerals are in Table 4. Although most minerals tended to be in higher concentration in the milk directly from the gland, only sodium was significantly higher (P<.05) in the milk obtained directly than in milk from the stomachs.

DISCUSSION

Milk composition of samples from stomachs of young cottontails within 5 min after the end of nursing was remarkably similar to the composition of milk directly from mammary glands of lactating cottontail rabbits. Although some differences in composition between the two methods of milk sampling were significant, they were not serious. Samples of milk from the stomachs of young provide a suitable source for analyses of milk constituents in cottontail rabbit milk.

Discrepancies in long-chain fatty acids of

TABLE 4. Major mineral analysis of cottontail rabbit milk.

Mineral	mg (%) (Mean ± SE) Stomach samples	Direct samples	Differences (P<.05)
Calcium	608 ± 33	693 ± 98	N.S.
Phosphorus	430 ± 18	478 ± 66	N.S.
Magnesium	43 ± 2	60 ± 6	N.S.
Potassium	162 ± 5	153 ± 24	N.S.
Chlorine	105 ± 7	142 ± 24	N.S.
Sodium	75 ± 4	146 ± 22	*

*Significantly different (P<.05).
N.S. = not significantly different.

milk fat between the two methods were probably a reflection of changes in the diets of the rabbits as season progressed, and not a difference due to methods of collection. In central Missouri, rabbits must change the selection of grass species from May to July because hot summer days cause several species of spring grasses to become dormant in July. The samples obtained directly from the mammary glands were harvested in July while those from stomachs of young were collected in April, May, or early June.

Sodium was significantly higher in samples of milk harvested directly than in the samples from stomachs of offspring. This was probably a result of the excessive trauma to the nipple that was required to express milk from the gland. In the sequence of normal nursing, this trauma is avoided because the young rely upon suction or vacuum at the nipple to remove the milk. Since there was no suction aiding in milk harvest, much more positive pressure than normal was exerted on the nipple to remove the milk. This tissue injury probably was responsible for an influx of sodium and perhaps chlorine into the milk.

Few studies have been concerned with composition of rabbits' milk. Several made use of milk obtained from the glands of the domestic rabbit *(Oryctolagus cuniculus)*. The composition of domestic rabbit milk compares closely with that of the Eastern cottontail rabbit (2, 4, 9). For example, Bergman and Turner (2) reported 12.2% fat, 10.4% protein, 1.84% lactose, and 2.0% ash as the milk composition of domestic rabbits.

Two references concerning the composition of cottontail rabbit milk were found. Jenness and Sloan (7) reported total solids at 36.1%, fat at 17.9%, total protein (casein plus whey) at 12.5%, lactose at 1.0%, and ash at 2.0%. All except the lactose compare closely with the study reported herein. This difference may be attributed to different methods used to analyze lactose. In a second study, one sample of milk was used to measure fatty acid composition of cottontail rabbit milk fat (6). Of particular note were the differences in amounts of lower chain fatty acids (C-8 and C-10) and in linoleic acid (C-18:2), the percentages being considerably higher for C-8 and C-10 and lower for C-18:2 in the earlier study than in our study.

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