GERMAN SHEPHERD DOG MILK COMPOSITION AND ITS CHANGES DURING LACTATION*

A. Dokoupilová¹, I. Svobodová¹, H. Chaloupková¹, L. Kouřimská², B. Dvořáková², R. Končel¹

 ¹Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Department of Husbandry and Ethology of Animals, Prague, Czech Republic
²Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Department of Quality of Agricultural Products, Prague, Czech Republic

Milk composition of nine lactating German Shepherd nursing females was studied. The experiment took place at the breeding facility of Police of the Czech Republic (breeding centre for service dogs in Domažlice) in days 2–30 of the females' lactation. Females were given a commercial granulated feeding mixture (starter category). Canine milk samples were analyzed using an infra-red instrument MilkoScan FT 120. Calculated mean values for colostrum (day 2 *postpartum*) and normal milk (days 4–29 *postpartum*) were: 23.86 and 24.63% for total solids, 8.14 and 7.22% for protein, 6.04 and 5.76% for casein, 10.22 and 11.32% for fat, 3.40 and 4.48% for lactose. German Shepherd milk showed almost no variation in composition after day 4 of lactation. Differences observed between colostrum and normal milk composition were statistically insignificant but not for lactose. No significant differences in density and other composition parameters between colostrum and normal milk were identified.

colostrum, normal milk, fat, protein, lactose, total solids



doi: 10.1515/sab-2016-0002 Received for publication on September 21, 2015 Accepted for publication on December 21, 2015

INTRODUCTION

Milk provides a complete diet for the new-born mammals. Milk composition varies widely across species with the stage of lactation (F arrell et al., 2006) and is influenced by multiple factors including the breed, health status of the mammal, feeding practices, and the time of milk samples collection *postpartum* (B ern a b u c c i et al., 2013).

Colostrum is the first milk produced after birth and is particularly rich in immunoglobulins, antimicrobial peptides, and other bioactive molecules, including growth factors. It is important for the nutrition, growth, and development of newborns and contributes to the immunologic defense of neonates (Playford et al., 2000). Bitches produce colostrum, which is more abundant in whey proteins (immunoglobulins) than mature milk, during 27-72 h immediately after birth (S e g a l i n i , 2008). Colostrum composition depends on the intensity that new-born mammals suck and varies with time after the birth, most significantly within 24 h after parturition (Bernabucci et al., 2013). Heddle, Rowley (1975) reported that the total immunoglobulin concentration in dog (mongrels) colostrum is 15 mg/ml on the day of parturition and 2-3 mg/ml on day 3 after parturition compared to 1-2 mg/ml for normal milk. However, the time of samples collection and rearing conditions were not described in this study. Costăchescu et al. (2011) compared the chemical composition of colostrum of German Shepherds, Caucasian Shepherds, Golden Retrievers, Labrador Retrievers, and West Terriers

^{*} Supported by the Ministry of the Interior of the Czech Republic, Project No. VG20132015133.

reared under the same conditions and with the same care. The samples of colostrum were collected from 10 females of each breed on day 2 after parturition and had the following average characteristics: fat 8.42%, protein 5.97%; water 75.77%, pH 6.0. German Shepherd colostrum was the richest in protein and the poorest in fat in comparison with other dog breeds. R ü s s e (1961) recorded that total protein decreases, whereas fat and lactose contents rise during the bitch colostral period.

After the colostral period, mammalian females produce mature milk consisting of an emulsion of fat and a suspension of casein micelles (caseins - the major milk proteins, calcium, phosphorus), all suspended in an aqueous phase which contains solubilized lactose, whey proteins, and some minerals (Bernabucci et al., 2013). In the past only a few studies focused on dog milk composition and its changes during lactation and widely differing values were reported. They were mostly published before 1990 (and not in English) (Abderhalden, 1899; Mundt et al., 1981; Kienzle et al., 1985). According to Oftedal (1984), dog milk contained 21-26% total solids; 8-12% fat, 7-10% protein on average. Scantlebury et al. (2000) observed that the milk of Labradors (n = 15) and Schnauzers (n = 6) from the Waltham Centre for Pet Nutrition fed by commercial diet contained on average 21.2% total solids, 8.23% crude protein, 8.11% fat, and 4.86% saccharide in days 24-30 of lactation with no difference among breeds. Also Rüsse (1961) recorded no differences in basic milk composition between breeds but Costăchescu et al. (2011) described differences in normal milk composition among five dog breeds. Oftedal (1984) reported the average contents of 22.7% total solids, 9.47% fat, 7.53% protein, and 3.81% saccharide for the milk of five Beagle bitches from a research colony with no changes in milk fat and protein concentration from day 7 to day 37 and with the increase of saccharide content from day 7 to day 30 of lactation. Luick et al. (1960) found out higher values (26% total solids, 9.8% protein, 13% fat) and Lönnerdal et al. (1981) lower values (5.31% protein, 4.66% fat, 4.81% saccharide) for Beagle milk. These differences may be explained by differences in analytical methods, in the number and collection time of samples or in feed composition of the observed bitches.

The studies focused on dog milk composition and its changes during lactation are essential for understanding the nutrition of neonatal dogs and for developing new puppy milk replacement formulas (L ö n n e r d a l et al., 1981). Although the replacers should be formulated to reflect the nutrient composition of dog milk, some of them contain either insufficient or excessive amounts of some nutrients (such as protein, fat, calcium, phosphorus), which can cause health problems in puppies (A d k i n s et al., 2001). The German Shepherd is the most frequent breed and the most commonly used dog for police and military purposes in the Czech Republic. But only little data about German Shepherd milk composition during lactation is available. Therefore, the aim of the study was to analyze the composition of dog milk of this breed and describe the changes during lactation.

MATERIAL AND METHODS

Experimental animals

Nine German Shepherd females were used in this study. The experiment took place in the breeding centre for service dogs (breeding facility of Police of the Czech Republic) in Domažlice during the first thirty days of the females' lactation. After this period the puppies were mainly given supplemental food. The females were given a commercial granulated feed mixture Royal Canin (sensible category) (28% protein, 18% fat, 2.8% fibre, 34.2% starch, 5.5% ash). The females were fed 2–3 times a day receiving 600 g of feed daily. The number of puppies in one litter was 6.43 ± 0.98 . The puppies were born in the period June 17^{th} –November 13^{th} .

Milk samples

Individual milk samples were taken by manual milking before noon feeding of females from randomly selected nipples of each female. Before milking the nipples were wiped with a warm wet cloth and then massaged. The milking took 2-7 min. Puppies were moved away from bitches during the sampling. Totally 54 samples were taken between 9:00 and 13:00 on the days indicated in Table 1. The first milk samples were taken 45–51 h after the puppies' delivery. Milk or colostrum were collected into standard 35-ml polypropylene milk sample vials with caps and immediately frozen to -18°C and delivered in cooling containers to the university laboratory for the analysis of basic milk parameters (fat, protein, lactose, total solids, casein, milk density). The samples were preheated to 40 C in a water bath before the analysis.

Instrumental analysis

Canine milk samples (each sample was measured twice) were analyzed using the infra-red apparatus MilkoScan FT 120 (Foss Electric, Hillerød, Denmark) calibrated by an external accredited laboratory using ten cow milk standard samples. The calibrated components in these standard samples were determined using reference methods recommended by Foss com-

Table 1. Parameters of German Shepherd milk during the first 30 days of lactation period

Parameter	Days of lactation					
	2	4	7–8	14-15	21-22	27–29
Milk density (g cm ⁻³)	1.027 ± 0.004	1.027 ± 0.003	1.032 ± 0.004	1.032 ± 0.005	1.034 ± 0.006	1.035 ± 0.002
Casein (%)	6.04 ± 1.15	4.90 ± 0.51	5.62 ± 0.49	5.83 ± 0.80	6.28 ± 1.04	6.15 ± 0.12
Protein (%)	8.14 ± 1.55	6.37 ± 0.48	7.08 ± 0.56	7.30 ± 1.00	7.83 ± 1.38	7.51 ± 0.25
Fat (%)	10.22 ± 1.49	10.29 ± 1.54	10.75 ± 1.20	11.47 ± 1.72	12.26 ± 3.01	11.84 ± 0.33
Lactose (%)	3.40 ± 0.40^a	4.14 ± 0.25^{b}	4.38 ± 0.24^b	4.43 ± 0.32^{b}	4.58 ± 0.29^{b}	4.84 ± 0.46^{b}
Total solids (%)	23.86 ± 1.99	22.18 ± 1.94	23.71 ± 1.50	24.85 ± 2.05	26.35 ± 3.30	26.05 ± 0.65

a,b means for lactose with the same subscript do not differ by more than significant level $\alpha = 0.05$; no significant differences concerning other parameters were found

pany (fat: Gerber method, protein: Kjeldahl method, lactose: Megazyme Lactose-Galactose enzymatic test kit, total solids and solids-non-fat: Heating Owen method at 102°C, freezing point: cryoscopic method).

Statistical methods

Means of eight milk samples taken on the same day of lactation were calculated with their standard deviations. The mean for the whole period of lactation except colostrum period was calculated as well. Milk component values were processed by the analysis of variance (ANOVA). The Scheffé's test was used to evaluate statistically significant differences between dog milk composition values during lactation. Statistical analyses were performed using SAS software, Version 9.3.

RESULTS

The results of the German Shepherd milk composition changes during the first 30 days of lactation expressed as the mean of 9 individual samples \pm standard deviation are given in Table 1. In our study the mean values for colostrum (day 2 *postpartum*) and normal milk (calculated from day 4 to days 27–29 *postpartum*) were: 23.86 and 24.63% total solids, 8.14 and 7.22% protein, 6.04 and 5.76% casein, 10.22 and 11.32% fat, 3.40 and 4.48% lactose, respectively. The changes of normal milk produced from day 4 *postpartum* were not significant.

However the protein content increased from day 4 till days 21–22 of lactation by 1.46%, and then slightly decreased (till days 27–29). The amount of casein as a proportion of total protein content increased from 64.2% (day 4) to 81.9% (days 27–29) in our experiment.

Similarly to protein, the fat content increased from day 4 till days 21–22 of lactation by 1.97%, and then decreased slightly by 0.32%.

In our study, the lactose content increased throughout the entire observation period with significant differences between colostrum and milk lactose content (P < 0.05).

There were no significant differences in density between colostrum and normal milk; the mean value of normal milk density was 1.032 g cm^{-3} .

DISCUSSION

This study is unique owing to two significant aspects: (1) sufficiently large sets of milk samples were collected from the same breed and (2) standardized breeding conditions were provided for the females by the breeding facility of Police of the Czech Republic in Domažlice – a unique institution of its kind in Europe. These conditions enabled us to obtain data on German Shepherd milk composition and its statistical evaluation.

The mean values for colostrum and normal milk noted in our study correspond with the values of Beagle bitches' milk composition reported by O ft e d a l (1984). Higher protein and carbohydrate (lactose) contents and lower total solids and fat content of colostrum were also noted in other dog breeds when compared with normal milk (S e g a l i n i , 2008; C o s t ă c h e s c u et al., 2011), although the variability of results is naturally large.

Unfortunately, there is very little data about dog colostrum composition in scientific literature. C o s t ă c h e s c u et al. (2011) reported very similar values concerning total solids and protein contents (24% and 8.1%), but lower values of fat (6.5%) in German Shepherd colostrum (2 days *postpartum*) if compared to the present experiment. In Beagle colostrum A d k i n s et al. (2001) noted higher content of protein (14%) and fat (13%) and lower content of lactose (2%) (1 day *postpartum*).

Like in our experiment, L ö n n e r d a 1 et al. (1981) described an increase in protein content throughout the lactation period of Beagles but with lower values: from 4.3% (days 0–10) to 5.31% (days 21–30). On the other hand, it was recorded that the protein content decreased from 7.5% on day 10 to 5.1% on

day 25 for German Shepherds (C ostăchescu et al., 2011). While in our experiment the amount of casein as a proportion of total protein content increased, A dkins et al. (2001) noted a higher percentage of casein (75.4%) in the early days of Beagle lactation and a lower percentage (69.5%) by day 28 (with non-significant differences).

Similarly to our data, the increase of fat content from day 10 till day 25 was reported for German Shepherds (Costăchescu et al., 2011) as well as Beagles (Lönnerdal et al., 1981). Oftedal (1984) and Adkins et al. (2001) observed the opposite tendency in the fat content of Beagle milk but with nonsignificant differences. Oftedal (1984) recorded that the fat content decreased from 10.9% (days 7-9) to 8.7% (days 22-23), and then increased to 9.16% (days 29–30). The author explained that the variation among studies may be the function of a sampling analytical bias or that this may represent the actual difference among dogs. Also some other factors like females' feed composition and energy intake in relation to their requirements as well as yield itself could be the reason for certain discrepancies in the existing literature.

In Beagles, the increasing values of lactose content during the first 30 days of lactation were confirmed by other authors (O f t e d a l, 1984; A d k i n s et al., 2001). L u i c k et al. (1960) noticed no differences in lactose content in Beagle milk measured on days 15 and 30 *postpartum*. However we noticed changes in lactose content during the first days of lactation in German Shepherds.

Like in other animals, differences in the density of colostrum and normal milk were insignificant. Density value is generally decreased by fat and increased by other non-fat solid compounds (proteins, lactose, and minerals). Our results therefore indicate that the effect of the fat content increase (by 0.07%) was compensated by the increase of non-fat solids (by 0.6%).

CONCLUSION

In conclusion, the present analysis of a reasonably large set of German Shepherd milk samples yielded novel unique data. German Shepherd milk showed almost no variation in composition after day 4 of lactation. Statistically significant differences in composition between colostrum and normal milk were observed only in the case of lactose. The obtained results enabled us to verify the published data about dog milk composition and provided new information on German Shepherd milk composition during lactation. Comparing the obtained results and the literature data on the composition of dog milk, it is evident that additional experiments focused on German Shepherd milk are needed. This is especially important in view of developing a puppy formula as a replacement for mother's milk.

REFERENCES

- Abderhalden E (1899): The relations of the rate of growth of the suckling to the composition of the milk in the dog, pig, sheep, goat and guinea pig. Zeitschrift für Physiologische Chemie, 27, 408-462. (in German)
- Adkins Y, Lepine AJ, Lönnerdal B (2001): Changes in protein and nutrient composition of milk throughout lactation in dogs. American Journal of Veterinary Research, 62, 1266– 1272. doi: 10.2460/ajyr.2001.62.1266.
- Anderson HD, Elvehjem CA, Gonce JE (1940): A comparison of the nutritive values of raw, pasteurized and evaporated milks for dog. Journal of Nutrition, 20, 433–443.
- Bernabucci U, Basiricò L, Morera P (2013): Impact of hot environment on colostrum and milk composition. Cellular and Molecular Biology, 59, 67–83. doi: 10.1170/T948.
- Costăchescu E, Hoha G, Fotea L (2011): Research regarding the lactating period of the bitch. Lucrări Științifice – Seria Zootehnie, 55, 180–183.
- Farrell Jr. HM, Malin EL, Brown EM, Qi PX (2006): Casein micelle structure: What can be learned from milk synthesis and structural biology? Current Opinion in Colloid & Interface Science, 11, 135–147. doi: 10.1016/j.cocis.2005.11.005.
- Heddle RJ, Rowley D (1975): Dog immunoglobulins. 1. Immunochemical characterization of dog serum, parotid saliva, colostrum, milk and small bowel fluid. Immunology, 29, 185–195.
- Kienzle E, Meyer H, Lohrie M (1985): The influence of carbohydrate-free rations with varying protein-energy relations on the fetal development and vitality of puppies as well as the lactic composition of female dogs. Journal of Animal Physiology and Animal Nutrition, 54, 76–77.
- Lönnerdal B, Keen CL, Hurley LS, Fisher GL (1981): Developmental changes in the composition of Beagle dog milk. American Journal of Veterinary Research, 42, 662–666.
- Luick JR, Parker HR, Andersen AC (1960): Composition of Beagle dog milk. American Journal of Physiology, 199, 731-732.
- Mundt H-C, Thomée A, Meyer H (1981): Energy and protein supply for suckling welpen through the bitch milk. Kleintierpraxis, 26, 353–360. (in German)
- Oftedal OT (1984): Lactation in the dog milk composition and intake by puppies. Journal of Nutrition, 114, 803–812.
- Playford RJ, Macdonald CE, Johnson W (2000): Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. American Journal of Clinical Nutrition, 72, 5–14.
- Rüsse I (1961): The lactation of the bitch. Zentralblatt für Veterinärmedizin, 8, 252–281. (in German)

Scantlebury M, Butterwick R, Speakman JR (2000): Energetics of lactation in domestic dog (*Canis familiaris*) breeds of two sizes. Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology, 125, 197-210. doi: 10.1016/S1095-6433(99)00175-0.

Segalini V (2008): Colostrum in dogs and cats. Point Vétérinaire, 39, 23–28. (in French)

Corresponding Author:

Adéla D o k o u p i l o v á , M.Sc., Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Husbandry and Ethology of Animals, Kamýcká 129, 165 21 Prague 6-Suchdol, Czech Republic, phone: +420 224 382 665, e-mail: dokoupilova@af.czu.cz