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Urea concentration in goat milk: importance of determination and factors of variability

Koncentracija uree u kozjem mlijeku: važnost određivanja i čimbenici varijabilnosti

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ABSTRACT

The optimal concentration of urea in cow's milk is known and is often used to assess the balance of energy and protein in diets. While in goat (and sheep) milk it is not controled nor defined. Main determinants of urea formation in milk are the amount of crude protein intake and the ratio betwwen protein and energy proportion in diet. Goat diet with excessive amount of crude protein and energy unbalanced diet cause the excess of nitrogenous substances in rumen along with release of ammonia and rise in concentration of urea in blood and milk, which adversely affects the production, milk coagulation propreties, environmental pollution and reproductive capabilities of goats. However, besides diet, there are other factors that influence milk urea concentration: breed, stage of lactation, parity, season, body mass, litter size, production and chemical composition of milk. Numerous studies conducted on cow milk included the influence of sources of variability listed above, while there are only few studies available for goat milk due to its less economic importance, seasonal polyesterity and different way of breeding and keeping goats. The aim of this paper is to comparatively and critically combine the previous research results on the importance of determining the milk urea concentration as well as on individual sources of variability of urea concentration in goat milk.

Keywords: goat milk, milk urea concentration, feeding, stage of lactation, parity, milk production

SAŽETAK

Optimalna koncentracija ureje u kravljem mlijeku je poznata i često se koristi za procjenu izbalansiranosti obroka energijom i proteinima, dok u kozjem (i ovčjem) mlijeku još uvijek nije definirana. Glavne odrednice stvaranja ureje u mlijeku jesu količina sirovih proteina u obroku i omjer proteinskog i energetskog dijela obroka. Hranidba koza s prekomjernom količinom sirovih proteina i energetski neuravnotežena hranidba uzrokuje višak dušičnih tvari u buragu, uz oslobađanje amonijaka i porast koncentracije ureje u krvi i mlijeku, što može nepovoljno utjecati na proizvodnju, koagulacijske osobine mlijeka, zagađenje okoliša i reproduktivne odlike koza. Međutim, osim hranidbe, postoje i drugi čimbenici koncentracije ureje u mlijeku: pasmina, stadij i redoslijed laktacije, vrijeme mužnje, sezona, tjelesna masa, veličina legla, proizvodnja i kemijski sastav mlijeka. Brojne studije provedene na kravljem mlijeku uključivale su utjecaj navedenih izvora varijabilnosti, dok je za kozje mlijeko dostupno svega nekoliko studija, vjerojatno zbog njegove manje ekonomske važnosti, sezonske poliestričnosti i različitog načina uzgoja i držanja koza. Cilj ovog rada je komparativno i kritički objediniti dosadašnje rezultate istraživanja o važnosti određivanja koncentracije ureje kao i pojedinim izvorima varijabilnosti koncentracije ureje u kozjem mlijeku.

Ključne riječi: kozje mlijeko, urea u mlijeku, hranidba, stadij i redoslijed laktacije, proizvodnja mlijeka

INTRODUCTION

Nowadays, there are more than one billion goats in the world and their number is continuously increasing. Following this global trend, there is also a steady increase in the population of dairy goats, mostly due to the growing demand for goat milk (Miller and Lu, 2019). Another reason for the increased interest in goat production may lie in the lower housing requirements due to their small body conformation, high fertility and early physiological maturity, seasonal polyestrous cycles and a large number of products with different use and purpose. Dairy goats are kept in different conditions, from extremely extensive on natural pastures where it is extremely difficult to balance nutrition and the assessment of feed intake is extremely difficult; to extremely intensive, where feeding is strictly controlled and rations consist of the basic voluminous part and the complementary concentrate part. Prerequisites for successful milk production are correct assessments of (a) the nutritional status of the flock, (b) the nutritional value of the feed and (c) the composition of diets in order to meet the needs of goats (Grbeša et al., 2005). It is known that urea concentration data in cow milk is often used to assess energy and protein balance in diets of dairy cows. However, there is very little data on the urea concentration in goat milk, primarily due to the lower economic importance of goats, i.e. goat milk (compared to cow milk), their seasonal polyestrous cycles and different ways of breeding and keeping (Goetsch, 2019).

Urea is a component of blood and other bodily fluids (milk, saliva, urine, gastric and intestinal juice) and is a common substance produced by the breakdown of body proteins by microorganisms (DePeters and Ferguson, 1992). This process allows fermentation and breakdown of organic matter into simpler chemical compounds such as ammonia and volatile fatty acids (Brun-Bellut, 1996 Bonanno et al., 2008). Excess protein in the ration (especially easily digestible) and the lack of energy feeds cause an excess of nitrogenous materials in the rumen, as well as a large release and resorption of ammonia and an increase in the milk urea concentration (Campanile et al., 1998). Due to the high correlation between the urea concentration in blood and milk, easier availability and smaller daily variations; the method of determining urea concentration in milk is preferred over its determination in the blood (Bed et al., 1997; Cannas et al., 1998; Schephers and Meijer, 1998). In order to produce as much milk as possible, breeders often feed the animals rations that are unbalanced in terms of supply of digestible proteins and energy. The consequence is a negative impact on production efficiency, milk processing properties, environmental pollution and animal fertility (Godden et al., 2001a; Mellado et al., 2004; Biswajit et al., 2011).

While the determination of milk urea concentration has practical application in monitoring the nutritional and reproductive status of dairy cows, it has not been systematically implemented in the production of goat (and sheep) milk. The reason lies in the fact that the interpretation of results is not fully standardized due to numerous influences such as nutrition, breed, stage of lactation, parity, season, milking time, body weight, production, chemical composition of goat milk, etc. (Schepers and Meijer, 1998; Giaccone et al., 2007; Abdouli et al., 2008; Goetsch, 2019). The objective of this study is to comparatively and critically consolidate previous results of research on the importance of determining the urea concentration in goat milk and to present the factors of its variability.

Urea synthesis

Of the total nitrogenous substances in milk, 95% are true proteins, and only 5% are non-protein nitrogenous materials, i.e. compounds that contain nitrogen in their molecule but are not proteins (urea, amino acids, creatine, creatinine, uric acid). As much as 60-100% of feed proteins that reach the body of ruminants are converted into ammonia. Rumen microorganisms use ammonia as a source of nitrogen and incorporate it into a microbial protein that, along with undigested food protein, goes into the small intestine, where it is broken down into simpler resorbable ingredients. If the microbial protein synthesis is slow, the excess of the formed ammonia in the rumen goes through the bloodstream to the liver, where a metabolic process converts it into urea. Therefore, it can be said that the mitochondrial liver cells are the main site of urea synthesis. From there, urea transported from the liver by the bloodstream to the kidney and is excreted into the urine (Figure 1).

In the case of kidney damage, or when animals are fed protein-rich diets, the concentration of urea in the blood plasma increases significantly (Mitin, 1974). When the urea concentration produced in the body exceeds the renal threshold, its concentration in the blood increases. Since urea synthesis seeks to remove excess nitrogen, i.e. to establish its balance, amino groups that are not needed in the biosynthesis of amino acids or other nitrogen compounds must be removed from the body, as ammonia is very reactive and as such toxic (Huntington and Archibeque, 1999). According to Symonds et al. (1981), the liver of dairy cows can successfully remove toxic ammonia up to a ruminal production of 12.0 g NH^3-N/h. Poisoning occurs at a production of 23.0 g NH^3-N/h and the cow lies helpless. If ammonia reaches a concentration of 2 to 4 mg/100 mL of blood in the peripheral circulation (posterior to the liver), the cow dies (Symonds et al., 1981). Excess protein in the diet, especially easily digestible one, and a lack of energy feeds will cause an excess of nitrogenous materials productions in the rumen, as well as a large release and absorption of ammonia increasing milk urea concentration (Campanile et al., 1998). The amino acid composition of proteins and nitrogen compounds for microbial synthesis in the rumen is not crucial because microbial synthesis and growth of rumen microorganisms are based on elemental substances of plant feeds such as easily soluble and digestible proteins and various nitrogen compounds (amino acids, nitrate, urea, ammonia). The key is to ensure a sufficient amount of carbohydrates for the rumen, in order to provide its microorganisms with a sufficient amount of energy to convert ammonia into a microbial protein (Vaga, 2017).

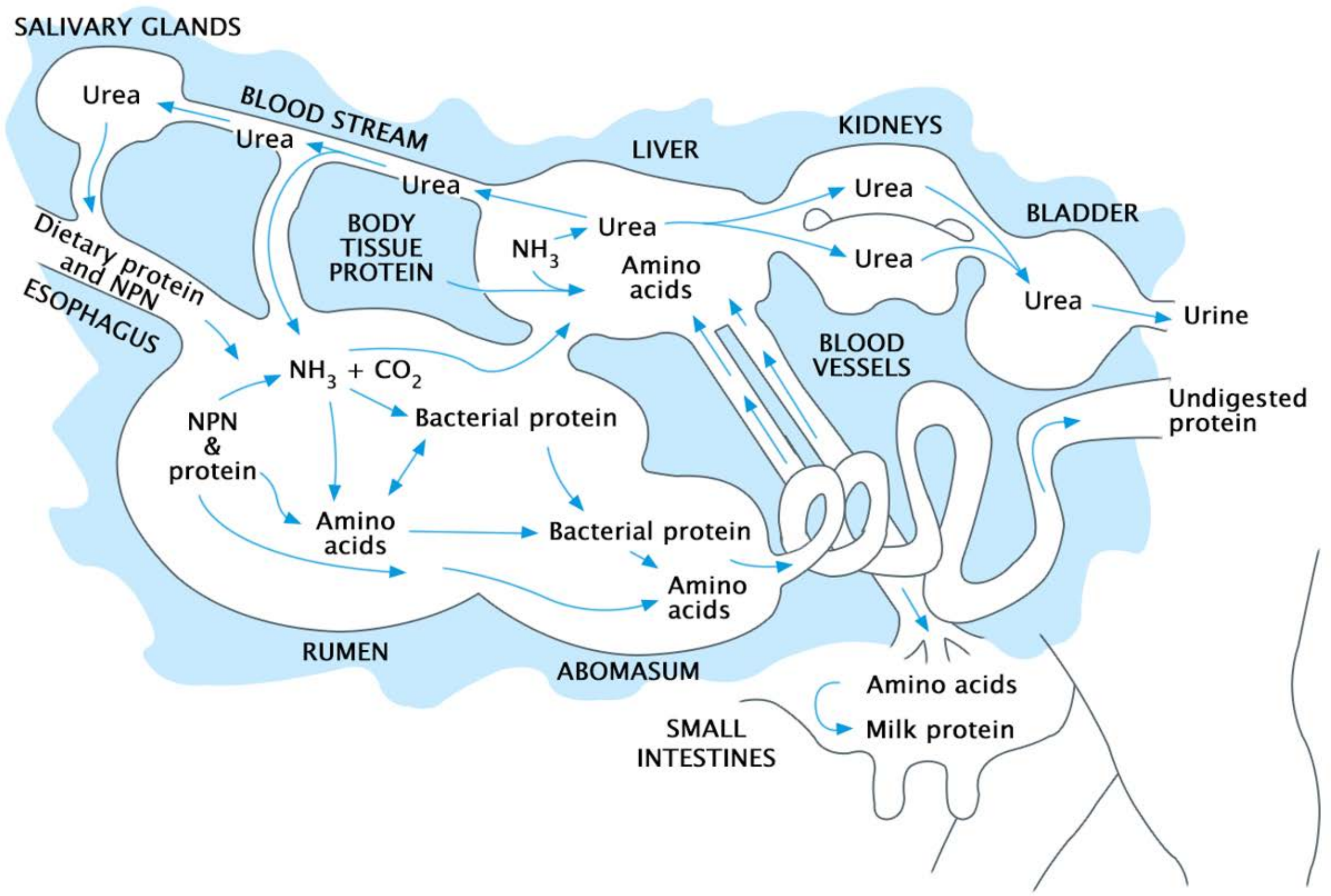

Figure 1. Nitrogen metabolism in ruminants (Source: Vaga, 2017, modified by Bendelja Ljoljić)

Determination of urea concentration in milk and blood

The blood flow through the kidneys of animals is constant and thus ensures a constant level of urea filtration (millilitres of blood filtered per minute), regardless of the urine volume. Most urea is excreted in the urine and if the urea concentration in the blood is high, more urea will be removed per minute compared to when the concentration is low, but the total amount of blood cleared of urea will be equal. After feeding, microbial degradation of feed protein will cause an increase in ammonia concentration in the rumen. Due to the transport of ammonia from the rumen into the blood and the subsequent conversion of blood ammonia to urea in the liver, urea concentration in bllod or milk will be increased. Since urea is a small molecule that diffuses easily into blood and milk, it is possible to explain the high correlation between urea concentrations in blood, milk and urine (Butler et al., 1996; Broderick and Clayton, 1997; Jonker et al., 1998; Nousiainen et al., 2004). A high and positive correlation between blood and milk urea concentrations in dairy cows was found by Roseler et al. (1993); Butler et al., (1996); Broderick and Clayton, (1997); in sheep Cannas et al. (1998), and in goats Cabiddu et al., (1999), Bava et al., (2001) and Sahoo and Walli, (2008). Analysing high number of milk samples Wittwer et al. (1999), found a slightly higher correlation (Figure 2) between the urea concentration in the blood and milk of cows (r=0.95). The higher urea concentration in the blood, the higher amount of urea will be excreted into urine and milk. Because of the high correlation between urea values in blood and milk, and due to easier availability and sample preparation, smaller daily variations and economic cost-effectiveness of the analysis, the method of determining urea concentration in milk has an advantage over its determination in the blood (Chládek and Máchal, 2004; Pazzola et al., 2011). The high correlation between urea concentration in milk and blood of dairy cows (r=0.84) provides a good basis for using urea concentration in milk to assess nitrogen excretion and efficiency of its utilization (Jonker et al., 1998; Nousiainen and et al., 2004) as well as for estimating ammonia emissions from manure into the soil (Powell et al., 2014).

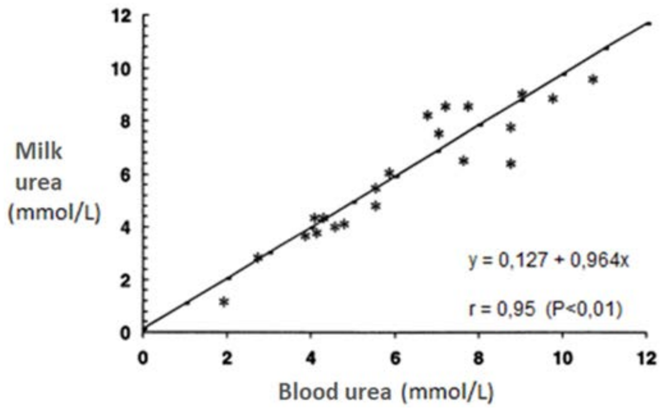

Figure 2. Relationship between milk urea and blood urea concentration (Source: Wittwer et al., 1999)

Individual and bulk milk samples

The urea concentration in the bulk milk sample should theoretically be equal to the weighted average of individual samples of the whole herd if individual milk weights, individual milk urea concentrations and urea concentrations are measured in the bulk sample without error at the same time (Biswajit et al., 2011). Nelson (1996), showed that the urea concentrations of bulk milk samples and the mean values of all individual samples differed by as much as 3.0 - 4.5 mg/100 mL (0.5 - 0.75 mmol/L). On the other hand, Broderick and Clayton (1997) and Jonker et al. (1998) suggest that urea concentration is not to be determined in individual milk samples but in a bulk sample of the whole herd. The advantages of this type of determination of the dairy herd nutritional status would lie in the reduction of labour costs and more economical sample analysis. However, there is doubt in the interpretation of these results, i.e. in the reliability of the results of urea concentration in the bulk sample being an indicator of the average urea concentration in the herd. Potential differences between the urea concentration of individual and bulk milk samples may result from inaccuracies in measuring individual milk weights, sample variance, analytical variance, and time effects since bulk and individual samples were not necessarily taken during the same milking time (Biswajit et al. 2011).

The importance of urea determination in milk

Determination of urea concentration is closely related to milk production efficiency (Santos et al., 2014), reproductive traits of goats (Godden et al., 2001a; Marenjak et al., 2004; Mellado et al., 2004; Butler, 2005; Biswajit et al. et al., 2011), milk processing properties (Pretto et al., 2013; Bland et al., 2015) and environmental pollution (Santos et al., 2014).

A balanced diet is considered to be the basis for efficient goat milk production. According to research, feeding costs account for about 50-70% of direct costs in goat milk production (Grgić, 2013; Kostelić, 2019). Therefore, a balanced feeding structure, balancing the actual needs of the individual, the use of alternative and cheaper sources of protein in order to reduce production cost, at the same time increasing the quantity and quality of produced milk (Santos et al., 2014). Urea serves as a good indicator of the goat's energy and protein supply. Therefore, it can be assumed that setting the limit value of urea concentration in goat milk would reduce feeding costs due to more rational protein (the most expensive dietary ingredient) intake, and feeding goats according to their actual needs would increase the farm's profit.

Biological mechanisms which would explain the association between urea concentration and reproductive traits of goats are still less known than in cows. Animal fertility is a major factor in the profitability of dairy flock. Animal fertility is affected by a number of factors, and nutrition management certainly plays a role in achieving satisfactory reproductive status. Excessive amounts of degradable proteins in the rumen may contribute to the factors resulting in a decrease in uterine pH during the luteal phase of embryonic development, which can consequently cause a decrease in fertility (Elrod and Butler, 1993). Early embryonic development requires appropriate conditions in the fallopian tube and uterus. High concentrations of urea or ammonia in the blood can be toxic to sperm, eggs, or the embryo, or can lead to the destruction of cilia in the fallopian tube (Moore and Varga, 1996). Feeding higher proportion of rumen non-degradable proteins (or bypass proteins) in the diet, the concentration of urea in milk and blood decreases, and the fertility of breeding animals improves. Several studies indicate a negative effect of high milk urea concentrations on the reproductive traits of dairy animals (Gustafsson and Carlson, 1993; Wittwer et al., 1999; Rajala-Schultz et al., 2001; Nourozi et al., 2010). It has been observed that the blood urea concentrations higher than 10 mg/100 mL (1.67 mmol/L) in cows increases the risk of miscarriage and consequently causes a low degree of conception (Mellado et al., 2004). However, other studies do not find negative effects of elevated milk urea concentrations on cow fertility (Melendez et al., 2000; Godden et al., 2001a).

One of the basic problems in milk processing is to achieve the appropriate suitability of milk for curdling, with the most important indicators being the milk coagulation properties (time, rate of curd formation and curd firmness). In contrast to goat milk, the influence of protein, casein, titratable acidity, calcium ion content, pH value, somatic cell count (SCC) and urea concentration on coagulation properties of cow milk, i.e. its processing properties in cheese production, are well studied and described. (Pretto et al., 2013; Bland et al., 2015). Investigating the relationship between the milk urea concentration and coagulation properties of goat milk (r- rennet coagulation time; k_{20}- time of curd firmness; and a_{30}- curd firmness), Bendelja Ljoljić (2018) found a statistically significant effect of urea concentration only on the rate of curd formation (k_{20}). However, due to the undetermined phenotypic correlation between the rate of curd formation (k_{20}) and the milk urea concentration, the same author found that their causal relationship cannot be confirmed. In contrast, a meta-analysis in cow milk (Bland et al., 2015) showed a significant effect of urea concentration on the rate of curd formation (k_{20}) and curd firmness (a_{30}) while Abilleira et al. (2010) reported a negative correlation between non-protein nitrogen and curd firmness in sheep milk. Studies in cow milk have shown that the milk urea concentration higher than 49 mg/100 mL (8.17 mmol/L) prolongs the clotting time of milk and reduces the curd firmness. Therefore, these cheeses contain more water (Martin et al., 1997). It is believed that increasing the milk urea concentration leads to partial dissociation of casein, calcium and phosphorus fractions into the soluble phase, prolonged milk clotting time, the formation of brittle curd, reduced acidification and irregular fermentation (Havranek et al., 2014).

The elevated concentration of urea, affects adversely not only the processing properties of milk, but nitrogen emissions present a major environmental problem in areas of intensive livestock production. The purpose of livestock production is to convert carbohydrates and proteins from animal into human food. However, only 5 to 30% of nitrogen from food is utilized in this way, and animals excrete the rest into the environment (Kohn et al., 2005). Nitrogen emissionfrom urea is an environmental problem because it is converted into atmospheric pollutants, namely: ammonia, which leads to the formation of acid rain; and nitrates that leak into surface and groundwater (Spek et al., 2013). Ammonia emissions are worrying because they can form smaller particles which cause asthma and other lung problems in humans (WHO, 2005). Nitrogen from urine can be dangerous to human health as it transforms into nitrates and thus enters drinking water (Wattiaux and Ranathunga, 2015).

Urea concentration values in goat milk

The physiological concentration of urea in goat milk is not defined, in contrast to cow milk in which it is present in the range of 10 to 30 mg/100 mL or from 1.67 to 5 mmol/L (Marenjak et al., 2004). Different studies have found different values of urea concentration in cow milk, which vary from 12 to 27 mg/100 mL, or from 2.0 to 4.5 mmol/L (Marenjak, 2007), while Young (2001) states that the recommended urea concentration in cow milk is 12 to 16 mg/100 mg (2 – 2.67 mmol/L).

Urea concentrations in goat milk based on research by different authors are presented in Table 1.

In order to assess meal balance, Brun-Bellut et al. (1991) reported a physiological range of urea concentration in goat milk of 28 - 32 mg/100 mL (4.67 - 5.33 mmol/L). According to Bendelja Ljoljić (2018), the urea concentration between 40 and 45 mg/100 mL (6.67 - 7.5 mmol/L). is a good indicator of a balanced diet for Alpine goats in terms of balanced metabolic microbial processes in the rumen, and consequently the smallest deviations in the produced daily amount, chemical composition and milk processing properties.

High milk urea concentration can be caused by excess protein in feed, not enough degradable carbohydrates in the rumen, excess protein that is not degraded in the rumen, insufficient water intake, detoxification processes that overload the liver or energy losses arising from keeping conditions (Biswajit et al., 2011). In contrast, the causes of too low concentration of urea in milk are insufficient levels of protein in feed, an excess of easily digestible carbohydrates, and an unbalanced intake of energy and protein (Jonker et al., 1998).

If the urea concentration is outside the physiological range of values, consideration should be given to how it affects milk production and whether energy and protein are balanced in the diet. Excessive consumption of protein in the meal leads to elevated levels of urea in the milk. If milk production is lower than expected due to the genetic potential of the animals, the reason can be found in possible excessive expectations for the level of milk production or in unbalanced meals with insufficient energy supply. If milk production is satisfactory and meets the expectations, but the urea concentration is not in the range of physiological values, it is necessary to check the composition of the mixture and examine whether the nutritional needs of the animal are met, but also whether the fodder is well mixed. The improperly mixture can lead to an inadequate distribution of nutrients in the mixture, and therefore some animals may be malnourished or overfed with a particular ingredient in the mixture (Babnik et al, 2004). The composition of feed mixtures can be significantly different depending on the method of chopping, cutting or grinding of components (Kohn, 2007). The urea concentration can also be significantly affected by grain processing. Namely, if the grain of wheat or some other dried components are dark brown in colour, it may contain a significant amount of bound protein that the animal cannot utilize. Also, if hay has been exposed to too much heat during canning, protein digestibility may be reduced and the diet may not contain sufficient amount of degradable protein which may result in low urea concentrations in milk (Kohn, 2007).

Table 1. Literature review of urea concentrations in goat milk

Milk urea ¹(mg/100 mL) ²(mmol/L)	Interpretation	Authors
¹17.6 – 21.6 ²2.93 – 3.6	Alpina goats fed a concentrate supplement with 11.5% crude protein	Min et al., 2005.
a) ¹21.7 ²3.62 b) ¹23.1 ²3.85	a) Jonica goats fed with a small amount in the rumen degradable proteins b) Jonica goats fed with a large amount in the rumen degradable proteins	Laudadio and Trufarelli, 2010.
¹22.5 ²3.75	Girgentana goats	Bonanno et al., 2008.
¹23.6 ²3.93	White short–hired goats	Kuchtík and Sedláčková, 2003.
¹28.87 ²4.81	Alpine goats fed a mixture of grains (wheat-30%, rye-30%, corn-30%, wheat bran-5% and soybeans-5%) with 13% crude protein and meadow hay with 11% crude protein	Antunović et al., 2017.
¹ 34.2 ²5.70	Mean value obtained from 5 conducted experiments with 13 different nutritional treatments	Rapetti et al., 2014.
a) ¹35.0 ² 5.83 b) ¹41.2 ²41.2 c) ¹48.1 ²8.02	a) Alpina goats fed a feed mixture with 14% CP b) Alpina goats fed a feed mixture with 16% CP c) Alpina goats fed a feed mixture with 18% CP	Bendelja Ljoljić, 2018.
¹39.2 - 41.2 ² 6.53 - 6.87	Feeding goats with 18-19% crude protein in dry matter of the meal	Rapetti et al., 2009.
¹ 40 - 45 ² 6.67 - 7.5	Girgentana goats fed with commercial concentrate	Giaccone et al., 2007.
a) ¹ 42.12 ² 7.02 b) ¹ 49.61 ² 8.27	a) Saanen goats fed a meal containing 11.4% crude protein b) Saanen goats fed a meal containing 17.8% crude protein	Superchi et al., 2007.
a) ¹ 52.4 ² 8.27 b) ¹ 56.2 ² 9.37 c) ¹ 56.2 ² 9.37	a) conventional production - alpine goat feeding alfalfa, meadow hay + 30-35% concentrate (corn, barley, wheat, sunflower) b) traditional production - alpine goat on pasture + 20% concentrate (soybean, corn, barley) + sudan grass, acacia leaves, ash, willow and vine c) organic production - alpine goats fed hay + 20% concentrate (corn, barley, oats and triticale)	Kučević et al., 2016.
a) ¹ 49.2 ² 8.2 b) ¹ 56.2 ² 9.37 c) ¹ 56.2 ² 9.37	a) goats stayed on pasture during the night b) goats stayed on pasture during the day c) goats stayed in the barn	Di Grigoli et al., 2009.
a) ¹ 62.4 ² 10.4 b) ¹ 59.4 ² 9.9 c) ¹ 59.3 ² 9.89 d) ¹ 54.4 ² 9.07	a) cross-bred goats fed with low undegradable protein b) cross-bred goats fed with low undegradable protein with molasses c) cross-bred goats fed with high undegradable protein d) cross-bred goats fed with high undegradable protein with molasses	Sahoo and Walli, 2008.

Factors influencing milk urea concentration

The concentration of urea in milk is influenced by a number of factors, nutrition being the most important. Also, there are numerous production and environmental factors such as breed and bodyweight of the animal, stage and number of lactation, milking time, season, production and chemical composition of milk and the number of somatic cells in milk (Giaccone et al., 2007; Abdouli et al., 2008). Variations in the milk urea concentration cannot be explained only by nutritional management or production and environmental factors, but by defining their joint impact. Of the total variations in the milk urea concentration, Arunvipas et al. (2003) attribute only 13.3% of the variations to non-nutritional factors, while Hojman et al. (2004) attribute 37% of individual variations to the same factors. Bonanno et al. (2008) claim that the dietary proportion of crude protein and neutral detergent fibres are the variables which gives the best explains on the variability of milk urea concentration.

Nutrition

The milk urea concentration depends on the level of consumption of crude dietary protein levels, the percentage of rumen degradable (RDP) and rumen non-degradable proteins (RUP), the ratio of energy and protein in the diet, the amount of easily digestible carbohydrates and water intake (Marenjak et al., 2004; Kohn, 2007; Bonanno et al., 2008; Biswajit et al., 2011). The high milk urea concentration is an indicator of high levels of ammonia in the rumen fluide, which can cause a number of problems such as overexploitation of energy to excrete nitrogen from the body, liver overload during the conversion of ammonia to urea and air pollution in the barn due to excessive nitrogen concentration in urine (Babnik et al., 2004).

The rumen degradable (RDP) and non-degradable (RUP) proteins are necessary nutrient source for rumen bacteria for protein synthesis. The RUPs, which are necessary to achieve a higher level of milk production, pass through the rumen unchanged, can or can not be digested in the small intestine. It is possible to ensure higher milk production by establishing a balance in the diet between RDP, RUP and non-fibrous carbohydrates (source of energy for rumen microorganisms), while at the same time the later component lowers protein content in the diet. According to the NRC (2007), only 45% of RDP is absorbed in the form of amino acids, and the remainder is excreted in the urine. For efficient utilization of nitrogen in the rumen, the microbial population needs adequate energy that mainly comes from sugar, starch and individual fibres. In contrast, 85% of RUP is absorbed, highlighting its importance for the efficient use of food protein.

The urea synthesis seeks to remove excess nitrogen from the body or to establish its balance, too much protein (especially easily digestible), with a simultaneous lack of energy feed in the meal results in excess nitrogen in the rumen. The consequence is a pronounced release and resorption of ammonia into the bloodstream and an increase in the milk urea concentration. The relationship between urea concentration and milk protein content is inverse, as lower urea concentration values are associated with higher food protein utilization. Namely, the low concentration of urea in milk is an indicator of low ammonia content in the rumen, which may be a warning sign of unfavourable conditions for the growth of microorganisms due to more difficult feed digestion in the rumen and poorer synthesis of microbial proteins (Marenjak et al., 2004). It is known that increased rumen intake of degradable proteins is responsible for the increase in urea concentration in milk and blood, and very little is used for milk protein synthesis (Roseler et al., 1993; Kampl and Stolla, 1995; Campanile et al., 1998; Marenjak et al., 2004; Akhtar et al., 2020). For example, Akhtar et al. (2020) found the highest concentration of urea in the blood of goats fed with a meal of 70% RDP compared to those fed with 60, 50, 40 and 30% RDP. Meng et al. (2016) showed that 2.5% more crude protein in the diet did not result in a higher proportion of protein in the milk, indicating the fact that excess ingested crude protein was excreted in the form of nitrogen in urine and faeces. They also point out that the ratio of energy and protein in a diet affects the breakdown and absorption of raw food proteins and their conversion into milk protein.

Reduced dietary protein intake may result in changes in the chemical composition of milk (Lee et al., 2012; Giallongo et al., 2015). The low milk urea concentration can be an indicator of unfavourable conditions necessary for the growth of rumen microorganisms. Consequently, digestion in the rumen is impaired and microbial protein synthesis is reduced (Marenjak et al., 2004).

According to literature data, a low concentration of ammonia in the rumen consequently cause lower milk urea concentrations than 20 mg/100 mL or 3.33 mmol/L (Marenjak et al., 2004; Min et al., 2005; Laudadio and Trufarelli, 2010). This is how the unfavourable conditions necessary for the growth of rumen microorganisms are created, whereby the synthesis of microbial proteins is reduced.

In a study conducted with Saanen goats in Italy fed 11.4% CP and 17.8% CP in the diet, the average milk urea concentration was 42.12 mg/100 mL (7 mmol/L) and 49.61 mg/100 mL or 7.27 mmol/L (Superchi et al., 2007). Studying the influence of the proportion of rumen degradable and non-degradable dietary proteins on the milk urea concentration, Laudadio and Tufarelli (2010) found that the increase in the urea concentration in goat milk occurred when feeding 17.5% CP, with a dominant share of rumen degradable protein. In contrast, in the extensive mode of goat keeping the increase in the proportion of crude protein in the supplementary portion of the diet has no effect the milk urea concentration (Cabiddu et al., 1999; Bava et al., 2001; Todaro et al., 2005; Bonanno et al., 2008). In addition, Santos et al. (2014) aimed to assess the degree of urea excretion and the synthesis of microbial proteins in Alpine goats fed a diet containing different sources of protein in the concentrate (soy, a meal composed of cotton seeds, cassava hay and alfalfa hay). These authors found the highest concentration of urea in the blood and milk in goats fed with soybeans and cotton seeds, which explains the better breakdown of protein in the rumen due to insufficient matching in carbon and nitrogen structure used by microorganisms during urea synthesis. In addition, the same authors showed that feeding goats a concentrate diet containing cotton seed results in higher urinary nitrogen excretion and negative nitrogen balance in the body than when the main source of diet protein composed by alfalfa hay which ensures more intensive microbial protein synthesis. An additional explanation for this conclusion lies in the fact that cotton seeds have more rumen degradable protein and less rumen non-degradable protein when compared to alfalfa hay.

Animal feeds that contain easily soluble and degradable proteins, such as green fodder, grass, wheat, barley, and similar grains, are not a good choice for achieving a high percentage of protein in milk (Feldhofer, 1997). The same author states that, in addition to rumen degradable proteins converted into microbial protein, using feeds with more stable proteins (corn silage, sunflower meal, rapeseed, dried hay, soybean husks), result in a larger amount of protein for degradation and better quality of amino acids required for milk tissue and protein synthesis entering the small intestine. With such nutrition, it is possible to improve the amount and quality of protein in milk. Babnik et al. (2004) showed that diets containing grass or grass silage predominantly are characterized by an increase in the milk urea concentration compared to diets rich in corn silage or hay. If the meal is rich in concentrate feeds, the rumen contents become acidic, the digestibility of forage feed and the synthesis of microbial protein are reduced, resulting in lower protein content in milk (Babnik et al., 2004). The milk urea concentration is also affected by other factors such as the amount of starch that cannot be broken down in the rumen and small intestine and fermented in the large intestine, so part of the nitrogen binds to microbial proteins excreted in the faeces. By feeding dairy animals a diet rich in rumen non-degradable starch, most of the nitrogen is excreted from the organism into the faeces, and only a small part in urine and milk (Jonker et al., 1998; Babnik et al., 2004). Therefore, at such feeding, and at the same concentration of ammonia in rumen fluide, a lower milk urea concentration can be expected.

Season

The season is an important factor in the variability of urea concentration in goat milk, daily production and chemical composition of milk (milk fat, protein and casein content). It is known that higher values of milk urea concentrations are determined during the summer months when the proportion of non-protein nitrogen fractions in milk increases (Carlsson et al., 1995; Hojman et al., 2004; Konjačić et al., 2010; Bendelja et al., 2011; Bendelja Ljoljić I sur., 2016; Bendelja Ljoljić, 2018). The cause of higher milk urea concentration in the summer season may be associated with the availability of voluminous feed. Carlsson et al. (1995) showed that the proportion of total nitrogen and true proteins (mostly casein) in cow milk decreases during the summer, while the proportion of non-protein nitrogen fractions in milk increases including a higher concentration of urea in summer. According to Moller et al. (1993), variations in cow milk urea concentration are associated with seasonal changes in grazing protein content and dietary energy components, and fresh grazing is supply to contain highly digestible proteins and a high energy-to-protein ratio. Hojman et al. (2004) also found direct influence of the season on the milk urea concentration, i.e. the highest values of urea in milk produced in summer.

The highest milk urea concentrations in the milk of Alpine goats during the summer period (41.94 - 48.55 mg/100 mL or 7 – 8.09 mmol/L) can be attributed to the overlap of the seasonal influence and the lactation stage, as a consequence of seasonal polyestrous cycles (Bendelja Ljoljić, 2018). Also, Kim et al., (2013) report the highest values of urea concentration in goat milk during the summer (30.14 mg/dL or 5.02 mmol/L) and the lowest in the spring (26.30 mg/dL or 4.38 mmol/L). The lower milk urea concentration in the spring may possibly be explained by reduced milk production as well as reduced ability to consume a total solids of the meal immediately after parturition. Giaccone et al., (2007) interpret seasonal variations in milk urea by physiological and nutritional conditions. The winter months (January, February and March) are characterized by higher levels of milk urea compared to the spring months (April, May and June), while the highest values were determined in July since Sicilian pastures are richer in soluble nitrogen in winter, but poorer in energy and neutral detergent fibres (NDF) than spring pastures. In contrast, Czarniawska-Zajec et al. (2006) reported a significantly higher concentration of milk urea (45.43 mg/100 mL or 7.57 mmol/L) in the winter months compared to the summer period (38.53 mg/100 mL or 6.42 mmol/L).

Stage of lactation

Several studies have found a close association between the urea concentration in goat milk and the lactation curve (Giaccone et al., 2007; Bendelja Ljoljić, 2018). During early lactation, the milk urea concentration is lowest due to animal's reduced dry matter intake immediately after parturition as well as due to a sudden increase in milk yield (Moore and Varga, 1996; Godden et al., 2001b; Rajala-Schultz and Saville, 2003). Assuming that the meal is balanced in terms of energy (fermentable carbohydrates) and protein (rumen degradable proteins), there is a decrease in the milk urea concentration in late lactation, as protein requirements of dairy animals are reduced and probably due to reduced supply of high protein plants on pasture. The change in urea concentration in cow milk parallel follows the lactation curve, i.e. the curve of daily milk production (Johnson and Young, 2003; Rajala-Schultz and Saville, 2003).

There is fairly few data on urea concentrations in goat milk related lactation stage. Most of the data on urea concentration in goat milk are related to its monitoring at 30-day intervals. For example, Kuchtík and Sedláčková (2003) found a maximum urea concentration of 30.92 mg/100 mL (5.15 mmol/L) in the milk of a local Czech goat breed on day 135 of lactation, while on day 258 of lactation it was only 19.16 mg/100 mL (3.19 mmol/L). According to Bendelja Ljoljić (2018), the determined concentration of urea in the milk of Alpine goats measuring 30-day interval was extremely variable and did not follow the lactation curve. The lower concentration of urea in goat milk in early lactation (39.31 mg/100 mL or 6.55 mmol/L), compared to the middle of lactation (45.72 mg/100 mL or 7.62 mmol/L), Bendelja Ljoljić (2018) considers a consequence of reduced ability to consume dry matter immediately after parturition and during a sharp increase in the lactation curve. Antunović et al. (2017) determined the lowest urea concentration in Alpine goat milk at the beginning and the highest at the end of lactation. In contrast to these results, Pazzola et al. (2011) did not find a statistically significant effect of lactation stage on urea concentration in the milk of Sarda goats, regardless of the daily amount of milk.

Parity

In general, increasing the number of lactations significantly increases the milk and blood urea concentration, regardless of the type of animal (Carlsson et al., 1995; Godden et al., 2001b; Arunvipas et al., 2003; Wood et al., 2003; Hojman et al., 2004; Doska et al., 2012; Bendelja Ljoljić, 2018). Basically, all these studies are conducted for cow while the number of studies of the influence of parity on the urea concentration in goat milk is negligible. The reason for the lowest milk urea concentration of urea during the first lactation can be explained by the fact that the young goats still have lower production capacity compared to adult goats. Also, lower milk urea concentrations are characteristic of all dairy animals during the first lactation, as they use amino acids from the diet for weight gain and development more efficiently, have reduced food consumption capacity and consequently less urea is synthesized in the liver and excreted in milk (Oltner et al., 1985). Giaccone et al. (2007) determined significantly lower milk urea concentrations in milk of the first lactation goats (41.09 mg/100 mL or 6.85 mmol/L) compared to lactating goats in the second and third lactations (45.03 and 44.43 mg/100 mL or 7.51 and 7.41 mmol/L). However, Jonker et al. (1998) and Doska et al. (2012) report higher urea values in the milk of younger cows, which may indicate an excess of crude protein in the diet.

Litter size

Very little research has been conducted to determine the effect of litter size on milk urea concentration; although some authors suggest that the number of suckling offspring is one of the factors of urea variability in goat or ewe milk (Sobiech et al., 2008; Bendelja Ljoljić, 2018). Trying to define the influence of lactation stage and litter size on the content of individual components of sheep milk, Sobiech et al. (2008) found a significant increase in milk urea concentration during lactation of sheep with two lambs compared to those with one lamb. The determined urea concentration, regardless of the lactation stage, was almost 6 mg/100 mL (1 mmol/L) higher in the milk of sheep with two lambs. At the same time, there was a decrease in the proportion of total protein in milk, which is probably related to protein catabolism during intensive milk synthesis. In contrast, Bendelja Ljoljić (2018) found a significantly higher milk urea concentration in Alpine goats with triplets in the litter than in those with one kid, and the difference found was almost 6 mg/100 mL (1 mmol/L). In addition, the urea concentration was twice as high in the milk of goats with three kids in the litter, and fed a mixture with 14% CP, then in those with one kid. The author finds the reason in the fact that goats that carry triplets must mobilize body reserves due to greater nutrient requirements during pregnancy. In order to replenish these spent reserves, they consume more feed and consequently, more urea is excreted from the body. However, Giaccone et al. (2007) did not report a significant effect of litter size on urea concentration in goat milk. Similar studies on the relationship between litter size and urea concentration, but in sheep milk, have been conducted by Brzostowski et al., (1995), El-Sherif and Assad (2001) and Sobiech et al. (2008), and all these studies found a higher concentration of milk urea in sheep with twins compared to the milk of sheep with only one lamb in the litter.

CONCLUSION

This paper critically combines data on the importance of determining the milk urea concentration and presents a literature review of its values in goat milk. Because of the large number of specific sources of variability, the optimal urea concentration in goat milk has not been defined yet. In general, the concentration of urea in milk depends on many factors, nutrition being the most important one. However, there are many other production and environmental (non-nutritional) factors such as breed, lactation stage, parity, season, animal husbandry, litter size, body weight, etc. Although the crude protein content in the diet explains most of the total variance in the goat milk urea concentration, it is by no means permissible to exclude the influence of non-nutritional factors. Therefore, it can be said that variations in milk urea concentration can only be explained by defining the combined influence of nutritional and non-nutritional factors.

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