

Changes in the concentrations of thyroid hormones in dairy sheep

Zuzanna Flis, Edyta Molik[#]

University of Agriculture in Krakow, Faculty of Animal Science, Department of Animal Nutrition and Biotechnology, and Fisheries, Al. Mickiewicza 24/28, 30-059 Kraków, Poland

SUMMARY

The aim of the research was to determine the changes in the concentrations of thyroid hormones (T3 and T4) in sheep during lactation. The experiment was conducted on 20 Polish Longwool ewes. Milking was begun after the lambs were weaned, on the 57th day of lactation. Sheep were milked twice a day (8:00 a.m. and 6:00 p.m.) until dry. The milk yield of each ewe was checked every 10 days. For determination of thyroid hormones, blood was collected every 28 days beginning at sunset, every 20 minutes for 6 hours. The average milk yield of the sheep was determined based on the individual yields on test days. The highest ($P \leq 0,01$) milk yield was obtained in the first month of milking, i.e. April ($0,48 \pm 0,018$ l). Milk yield decreased as lactation progressed. The concentration of hormone T3 was significantly higher ($P \leq 0,01$) in the first and third months of lactation ($0,72 \pm 0,02$ ng/ml in April and $0,74 \pm 0,02$ ng/ml in June) than in September ($0,55 \pm 0,01$ ng/ml). The T3 concentration decreased in successive months of lactation. In contrast, the concentration of the T4 hormone increased with the progression of lactation. Significantly ($P \leq 0,01$) the lowest concentration of T4 was found in the first and second month of milking ($51,7 \pm 9,8$ ng/ml, $48,8 \pm 7,4$ ng/ml). The highest T4 concentration in lactating sheep was noted in August ($60,55 \pm 10$ ng/ml). The research showed that the secretion of T3 decreases with milk yield, while secretion of T4 shows an upward trend as lactation progresses. Therefore thyroid hormones modulate secretion of milk in sheep.

KEY WORDS: sheep, thyroid hormones, lactation, milk

INTRODUCTION

Lactation is the process of milk production and secretion by the mammary glands of female mammals (Lakhani et al., 2017). In sheep, it is the last stage of the reproductive cycle (Błasiak and Molik, 2014). Milk is a source of nutrients, immune substances, and other valuable bioactive substances essential for the proper development of the young (Ferreira et al., 2013). Milk production

[#]Corresponding author e-mail: rzmolik@cyf-kr.edu.pl

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is associated with considerable energy expenditure and takes place via transport of material from the blood to the udder (Kelly and Bach Larsen, 2010). In this process, thyroid hormones (triiodothyronine and thyroxin) are responsible for regulation of metabolic processes that control the energy balance in the body (Cheng et al., 2010) and body weight (Iwen et al., 2013). They also take part in the neuronal development of the foetus, the functioning of the cardiovascular system, and the initiation and maintenance of lactation (Todini, 2007; Błasiak and Molik, 2014; Ortiga-Carvalho et al., 2016; Carvalho and Dupuy, 2017, Molik et al., 2019). The initiation and maintenance of lactation in seasonal animals requires the presence of numerous physiological and environmental factors. Therefore the aim of the study was to determine the changes in thyroid hormones in sheep during lactation.

MATERIAL AND METHODS

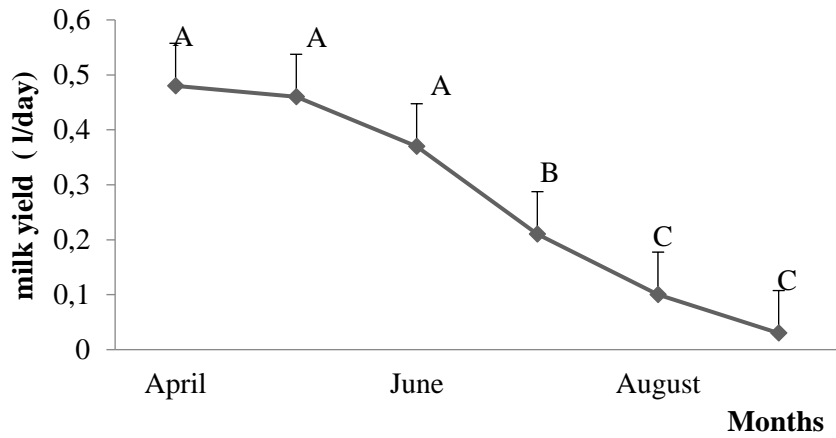
The study was conducted on 20 Polish Longwool ewes of similar body weight ($60 \text{ kg} \pm 5 \text{ kg}$) and age (4-5 lactations). During the experiment the ewes were housed indoors with access to runs. The study was carried out with the approval of the Local Ethics Committee. The oestrous cycle of the ewes was synchronized by the Chrono-Gest method. For this purpose, polyurethane sponges impregnated with 40 mg of Cronolone (Intervet, Netherlands) were administered intravaginally and left for 14 days. On the day the sponges were removed, the sheep were given 500 IU PMSG (Serogonadotropin, Biowet, Drwalew). Oestrus began about 48-72 hours after administration of PMSG. The duration of oestrus was additionally verified using a teaser ram. Ewes were mated from 10 to 15 September. Lambing took place and lactation began in mid-February. Lambs were reared with their mothers for 56 days. The diet of the ewes was adjusted to their physiological state: up to the end of the fourth month of gestation they were fed according to the principles and standards of the National Research Institute of Animal Production (1993). From the fifth month of gestation until dry-off, the sheep received a uniform diet in the form of a pelleted compound feed and meadow hay *ad libitum*. Milking was begun on the 57th day in milk, after the lambs were weaned. The ewes were milked twice a day (at 8:00 a.m. and 6:00 p.m.) until dry, using an Alfa-Laval direct-to-can milk machine. Individual milk yield was recorded every 10 days, as the sum of the amounts of milk obtained from morning and evening milking. The ewes were milked from April to September. Milk yield in each month was calculated as the average from the yield on the test days. For determination of thyroid hormones, every 28 days (at the end of the month) blood was collected from the ewes using a catheter placed in the jugular vein. To accustom the animals to the intravenous cannula and eliminate the stress factor, the catheters were inserted 5 hours before blood was taken. Blood was taken from the ewes every 20 minutes for 6 hours. Hormones T3 and T4 were determined by radioimmunoassay (RIA) (Cisbio) as described by Kokot and Stupnicki (1985). The data pertaining to changes in milk secretion and thyroid hormones were analysed by ANOVA using the Kruskal-Wallis test.

RESULTS AND DISCUSSION

The study showed that in the first month of milking the average daily milk yield of the sheep was $0,48 \pm 0,018 \text{ l}$ and was significantly ($P \leq 0,01$) higher than in June and in the subsequent months. In the second month of milking (May), milk yield was $0,46 \pm 0,017 \text{ l}$ and was significantly higher ($P \leq 0,01$) than the milk yield in subsequent months. In June, milk yield was $0,37 \pm 0,012 \text{ l}$ and was significantly lower ($P \leq 0,05$) than in April and May. In the fourth month of milking (July), milk

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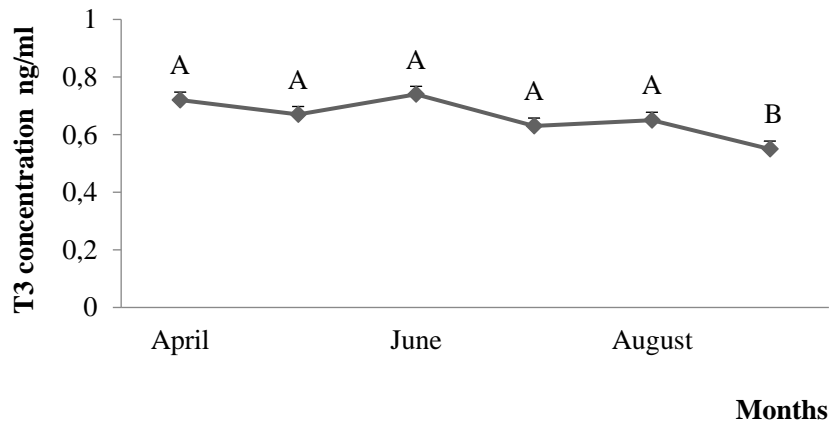
yield was $0,21 \pm 0,011$ l and was significantly lower ($P \leq 0,01$) than the previous yields. As lactation progressed, milk secretion decreased. The lowest ($P \leq 0,01$) milk yield was noted in the last two months of milking ($0,1 \pm 0,011$ l in August and $0,03 \pm 0,001$ l in September) (Fig. 1.).



A, B, C – means with different letters differ significantly at $P < 0,01$

Fig. 1. Changes in milk yield of sheep during lactation

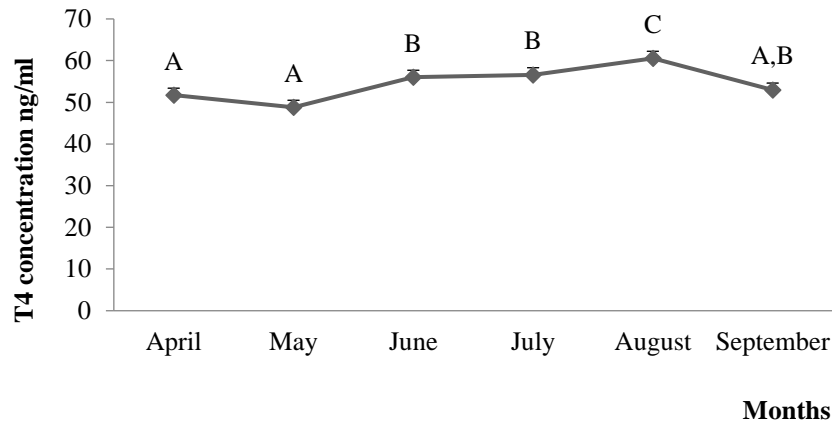
The results showed significantly ($P \leq 0,01$) the highest concentration of the hormone T3 in the first and third month of milking ($0,72 \pm 0,02$ ng/ml in April and $0,74 \pm 0,02$ ng/ml in June). As lactation progressed, the concentration of T3 decreased. In the last month of milking, significantly ($P \leq 0,01$) the lowest T3 concentration ($0,55 \pm 0,01$ l) was observed in comparison to the previous months (Fig. 2.).



A, B, C – means with different letters differ significantly at $P < 0,01$

Fig. 2. Changes in triiodothyronine (T3) concentration in sheep during lactation

The results pertaining to the T4 profile showed significantly ($P \leq 0,01$) the lowest concentration of this hormone in the first two months of milking ($51,7 \pm 9,8$ ng/ml in April and $48,8 \pm 7,4$ ng/ml in May). In June and July the T4 concentration increased, amounting to $56,4 \pm 8,2$ ng/ml and $56,6 \pm 8,3$ ng/ml, respectively. The differences were significant ($P \leq 0,01$) in comparison to the first two months of milking. The highest ($P \leq 0,01$) concentration of T4 was noted in August ($60,55 \pm 10$ ng/ml). In the last month of milking, the T4 concentration significantly ($P \leq 0,01$) decreased relative to the previous month and was $52,93 \pm 8,7$ ng/ml (Fig. 3.).



A, B, C – means with different letters differ significantly at $P < 0,01$

Fig. 3. Changes in thyroxin (T4) secretion in sheep during lactation

The study showed that milk yield decreased as lactation progressed. The results are confirmed by previous research on changes in milk secretion during lactation (Andrade et al., 2008; Bass, 1989; Fuertes et al., 1998; Armaou et al., 2018). In sheep, as seasonal animals, milk yield is also dependent on day length (Molik et al., 2007, 2009, 2013). In the present study, milk yield decreased in successive months of lactation, due in part to changes in the length of the day from April to September.

Previous research has shown that triiodothyronine modulates milk production in sheep by inhibiting transcription of the prolactin gene. This is due to blocking of the signal transducer and transcription activator 5a (STAT5a), which functions as a mediator in the prolactin signalling pathway in the mammary gland (Campo Verde Arboccó et al., 2015). A low level of thyroid hormones in the blood has been observed in sheep at the start of lactation, gradually increasing as lactation progressed (Mitin et al., 1986). The present study showed an increase in the T3 concentration in the third and fifth month of milking, while the lowest concentration was observed in the final month of lactation, when the days were short. A similar pattern was shown in an in vitro study on thyroid explants. The concentration of triiodothyronine was shown to be higher in the summer and declined when the days became shorter (Klocek-Górka et al., 2010). The thyroxin profile, on the other hand, showed the reverse tendency to triiodothyronine. As lactation progressed from April to August, the concentration of thyroxin increased. Previous research has shown that the

thyroxin concentration increases over the course of lactation in sheep, but decreases in goats (Błasiak and Molik, 2014). In the present study, the lowest T4 level was observed in May, which seems to be in agreement with in vitro research showing a lower concentration of this hormone in the spring, when the day is longer, and higher concentrations when the days are shorter (Klocek-Górka et al., 2010).

To sum up, the study showed that as lactation progresses, the yield of milk and the concentration of triiodothyronine decreases, while the concentration of thyroxin increases. Changes in the concentrations of thyroid hormones (T3 and T4) affect milk yield in sheep during lactation, especially in seasonal sheep. This suggests the need for further research aimed at determining the role of metabolic hormones in the initiation and maintenance of lactation.

REFERENCES

1. Andrade B. R., Salama A. A. K., Caja G., Castillo V., Albanel, E., Such X. (2008). Response to Lactation Induction Differs by Season of Year and Breed of Dairy Ewes. *Journal of Dairy Science*, 91(6): 2299-2306. doi:10.3168/jds.2007-0687
2. Armaou E., Simitzis P., Koutsouli P., Zoidou E., Massouras T., Goliomytis M., Bizelis I., Politis I. (2018). The effect of extended milking intervals (24, 48 and 72 h) on milk yield, milk composition, mammary physiology and welfare traits in dairy ewes. *Journal of Dairy Research*, 1-7, doi: <https://doi.org/10.1017/S002202991800047X>
3. Bass J. (1989). Effect of litter size, dietary protein content, ewe genotype and season on milk production and associated endocrine and blood metabolite status of ewes. *Animal Breeding Abstracts* 58: 275
4. Błasiak M., Molik E. (2014). Rola hormonów tarczycy w regulacji sezonowego cyklu reprodukcyjnego u małych przeżuwaczy. *Roczniki Naukowe Zootechniki*, 41: 3-8
5. Campo Verde Arboccó F. V., Sasso C. L., Nasif D., Belén Hapon M., Jahn G. A. (2015). Effect of hypothyroidism on the expression of nuclear receptors and their co-regulators in mammary gland during lactation in the rat. *Molecular and Cellular Endocrinology*, 412: 26-35, <https://doi.org/10.1016/j.mce.2015.05.026> Get rights and content
6. Carvalho D. P., Dupuy C. (2017). Thyroid hormone biosynthesis and release. *Molecular and Cellular Endocrinology*, 458: 6-15. doi: 10.1016/j.mce.2017.01.038
7. Cheng S. Y., Leonard J. L., Davis P. J. (2010). Molecular aspects of thyroid hormone actions. *Endocrine Reviews*, 31: 139-170. <https://doi.org/10.1210/er.2009-0007>
8. Ferreira A. M., Bislev S. L., Bendixen E., Almeida A. M. (2013). The mammary gland in domestic ruminants: A systems biology perspective. *Journal of Proteomics*, 94: 110-123. <https://doi.org/10.1016/j.jprot.2013.09.012>
9. Fuertes J. A., Gonzalo C., Carriedo J. A., San Primitivo F. (1998). Parameters of Test Day Milk Yield and Milk Components for Dairy Ewes. *Journal of Dairy Science*, 81(5): 1300-1307. [https://doi.org/10.3168/jds.S0022-0302\(98\)75692-9](https://doi.org/10.3168/jds.S0022-0302(98)75692-9)
10. Iwen K. A., Schroder E., Brabant G. (2013). Thyroid hormone and the metabolic syndrome. *European Thyroid Journal*, 2: 83-92. doi:10.1159/000351249
11. Kelly A. L., Bach Larsen L. (2010). Milk biochemistry. Improving the Safety and Quality of Milk, 3-26

12. Klocek-Gorka B., Szczesna M., Molik E., Zieba D. A. (2010). The interactions of season, leptin and melatonin levels with thyroid hormone secretion, using an in vitro approach. *Small Ruminant Research*, 91(2-3), 231-235. doi: 10.1016/j.smallrumres.2010.03.005
13. Kokot F., Stupnicki R. (1985). *Metody radioimmunologiczne i radiokompetycyjne stosowane w klinice*. PZWL, Warszawa
14. Lakhani P., Thakur A., Kumar S., Singh P. (2017). Artificial Induction of Lactation in Bovines - Scope and Limitations. *International Journal of Livestock Research*, 7: 102-112. doi: 10.5455/ijlr.20170324031735
15. Mitin V., Mikulec K., Karadjole I. (1986). Thyroid hormones and insulin concentration in sheep. *Veterinarski Arhiv*, 55: 573-575. doi: 10.1017/S0022029912000118
16. Molik E., Misztal T., Romanowicz K., Wierzchoś E. (2007). Dependence of the lactation duration and efficiency on the season of lambing in relation to the prolactin and melatonin secretion in ewes. *Livestock Science*, 107: 220-226. <https://doi.org/10.1016/j.livsci.2006.09.013>
17. Molik E., Misztal T., Romanowicz K., Zięba D., Wierzchoś E. (2009). Changes in growth hormone and prolactin secretion in ewes used for milk under different photoperiodic conditions. *Bulletin of the Veterinary Institute in Pulawy*, 53: 389-393
18. Molik E., Pasternak M., Błasiak M., Misztal T., Romanowicz K., Zięba D. (2013). The effect of the diversified signal of melatonin on milk yields in seasonally breeding sheep. *Archiv fur Tierzucht/Archives Animal Breeding*, 93: 924-932
19. Molik E., Flis Z., Staroń M., Hell K. (2019). Znaczenie hormonów tarczycowych w laktacji u owiec. *Roczniki Naukowe Zootechniki*, T. 46, z. 1 3-9
20. Ortiga-Carvalho T. M., Chiamolera M. I., Pazos-Moura C. C., Wondisford F. E. (2016). Hypothalamus-Pituitary-Thyroid Axis. *Comprehensive Physiology*, 1387-1428
21. Todini L. (2007). Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. *Animal*, 1(7): 997-1008. doi: 10.1017/S1751731107000262