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Metabolic hormone profiles pre- and post-fattening in sheep breeds in Latvia with varying feed efficiency

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Abstract. In sheep production, livestock fattening constitutes a major source of income, with overall economic efficiency largely determined by feed efficiency (Fe). Feed efficiency, in turn, is influenced by the hormone-regulated physiological state of the animal. Monitoring hormone levels as biomarkers can significantly optimise the lamb fattening process, improving outcomes. The study aims to analyse levels of hormone IGF-1, insulin, total T4, ACTH, haematocrit (HCT), haemoglobin (Hb), and glucose in sheep breeds raised in Latvia at different ages or fattening periods and corresponding Fe indicators. Blood samples and phenotypic data were collected from 76 and 92 intensively fattened lambs at ~81 and ~150 days of age, respectively, with ~60% representing the Latvian Dark-Head breed. Post-fattening levels of IGF-1, insulin, haematocrit, and haemoglobin were statistically higher, while glucose was elevated pre-fattening. IGF-1 and insulin varied significantly across five and three breeds; HCT and Hb levels differed significantly in one breed each. Pre-fattening IGF-1 and glucose levels correlated with feed efficiency traits. Regression analysis showed that pre-fattening hormone levels explained 24.7% of FCR, 22.8% of RGR, and 31.6% of KR variance.
Results demonstrate breed-specific hormonal differences in Latvian sheep and indicate the potential of hormone levels as biomarkers for assessing and optimising feed efficiency, emphasizing the need to study hormone levels across breeds and at different fattening stages.

Key words: Breeding, fattening, feed efficiency, metabolic hormones, Latvian sheep.

INTRODUCTION

The assessment of key haematological and biochemical parameters provide objective insights into the physiological status of animals at the time of sampling and may serve as an indicator of superior individual quality when such parameters are validated as biomarkers (Vojta et al., 2011). Although reference ranges for the most relevant important haematological and biochemical indicators have been established (Dias et al., 2010; Pugh & Baird, 2012; Fish & Pugh, 2021; Amokrane-Ferrah et al., 2022), studies have demonstrated that average values often differ across sheep breeds (Vojta et al., 2011) largely due to physiological variability influenced by age, body weight, and feeding regimen (Abdelsattar et al., 2022). Reference intervals typically reflect values observed in 95% of a healthy population, meaning that approximately 5% of individuals may present values outside these established ranges (Seixas et al., 2021).

Feed efficiency (Fe), a key indicator of fattening performance, is influenced by factors such as body composition, nutrient digestibility, and metabolic activity (Zhang et al., 2017). Several metrics are commonly used to evaluate feed efficiency, including feed efficiency (FE), feed conversion ratio (FCR), relative growth rate (RGR), Kleiber ratio (KR), residual feed intake (RFI), residual weight gain (RWG) and residual intake and body weight gain, reduced average production cost ratio (RIG) (Berry & Crowley, 2013; Trapina et al., 2023). Enhancing feed efficiency improves production economics and reduces the environmental footprint of livestock farming (Hu et al., 2022).

The endocrine system influences feed efficiency through the regulating of feed intake and nutrient utilisation (Richardson et al., 2004). Hormonal profiles related to nutrient metabolism vary among lambs from different breeds and are associated with differences in feed efficiency and meat quality (Zhang et al., 2017).

The gastrointestinal tract and its associated endocrine glands secrete hormones that play key roles in regulating feed intake and nutrient utilisation, including insulin, glucose, and insulin-like growth factor 1 (IGF-1). Prior research indicates systemic insulin levels in low-feed efficiency steers exceed those in high-feed efficiency steers (Richardson et al., 2004). Additionally, plasma levels of thyroxine (T4) and adrenocorticotropic hormone (ACTH) have been reported to be lower in high-efficiency cows relative to their low-efficiency counterparts (Walker et al., 2015).

Analysing of haematological and biochemical parameters in intensively fattened lambs of six sheep breeds raised in Latvia revealed significant interbreed differences in average values, despite identical feeding conditions (Trapina et al., 2023).

In sheep breeding, selection animals for meat production or breeding purposes is challenging at birth or shortly after weaning, as it requires early prediction of the animal's productive potential. Understanding the relationship between post-weaning haematological and biochemical indicators and subsequent feed efficiency may enable the development of predictive algorithms for practical selection strategies.

The study aims to evaluate the levels of nutrient metabolism-related hormones in lambs of Latvian sheep breeds prior to intensive fattening, and to compare these values with previously reported post-fattening hormone profiles (Trapina et al., 2023) Additionally, the study aims to assess the relationship between these hormone levels and feed efficiency indicators to identify potential early predictors of fattening performance.

Scientifically based knowledge of breed-specific hormonal profiles and their associations with feed efficiency traits can serve as a cost-effective and rapid selection tool within individual herds. By identifying key haematological and biochemical indicators as predictive biomarkers prior to fattening, it becomes feasible to select animals with superior feed efficiency for breeding. This approach enables the systematic improvement of sheep breeds and overall herd productivity.

MATERIALS AND METHODS

Animals' group of study

In accordance with the national breeding program requirements for sheep breeds raised in Latvia (LAAA, 2022), the progeny of certified sire rams are annually selected and evaluated to assess sire performance. A total of 84 lambs born as twins, triplets or quadruplets from six breeds: Latvian dark-head (LT; 61.91%), Merinolandschaf (MLS; 4.76%), Île de France (IF; 14.29%), Charollais (CH; 4.76%), Dorper (DOR; 7.14%) and Texel (TEX; 7.14%), were included in controlled fattening at the ram breeding evaluation station 'Klimpas' in collaboration with the association 'Latvian Sheep Breeders Association' from March to October 2023. The proportion of breeds corresponds to the distribution of purebred sheep breeds raised in Latvia.

Biochemical analysis

At the onset of the fattening period following an adaptation phase of 1 to 2 weeks the body weight of each lamb was recorded (average age of lambs: 81.67 ± 9.20 days), and blood samples were collected from the jugular vein for haematological and biochemical analyses. The analysed parameters included insulin-like growth factor 1 (IGF-1), insulin, total thyroxine (T4), adrenocorticotropic hormone (ACTH), haematocrit (HCT), haemoglobin (Hb), and glucose. All blood samples were obtained in the morning, prior to midday, and processed in a certified laboratory.

Intensive fattening and its indicators

Detailed information on feeding conditions, dietary composition during intensive fattening, phenotypic measurements, and the calculation of efficiency indicators has been described in previous studies (Trapina et al., 2023; Trapina et al., 2024a; 2024b) and remained consistent throughout the current investigation.

All offspring: three to four lambs per sire were housed together in a pen measuring approximately 4m², each equipped with a loose silo for concentrate and a slatted silo for hay, allowing ad libitum access to both feed types. Straw was used as bedding material. After each fattening batch, pens were thoroughly cleaned and disinfected. The facility was equipped with slotted walls and windows fitted with insect-proof nets to ensure natural ventilation. These housing conditions complied with established animal welfare standards throughout the study.

The lambs were fed a formulated concentrated feed supplemented with hay, mineral feed, salt licks. They had unrestricted access to water via automatic drinkers, in accordance with the intensive fattening control protocol developed by LAAA specialists (Šenfelde et al., 2020). All animal care procedures adhered to established animal welfare regulations.

The fattening period lasted on average 65.19 ± 13.10 days, ranging from 42 to 98 days.

Lamb live weight was measured before and after intensive fattening period using calibrated and certified electronic scales with an accuracy of ± 0.01 kg. The fattening was concluded once the average final body weight of all offspring from a single sire ram, housed within the same pen, reached at least 45–50 kg, in accordance with the control fattening protocol.

Calculated values for average daily gain (ADG), dry matter intake (DMI), and live weight were used to derive several feed efficiency metrics, including feed efficiency (FE), feed conversion ratio (FCR), relative growth rate (RGR), and Kleiber's ratio (KR), as well as residual-based indicators such as residual feed intake (RFI), residual weight gain (RWG), and residual intake and body weight gain (RIG). These calculations were performed using formulas described in previous studies (Berry & Crowley, 2012; Lima et al., 2017; Trapina et al., 2024a).

Statistiscal analyses

Haematological and biochemical parameters measured in lambs at 81 days of age, prior to the fattening period, were compared, across breeds by stratifying the animals according to breed classification.

Pre-fattening haematological and biochemical parameters of the current lamb group were also compared with post-fattening values obtained from a separate group of 76 lambs, previously fattened under identical feeding and management conditions, at approximately 150 ± 13.44 days of age (Trapina et al., 2023). The breed distribution in the post-fattening group was proportionally similar to that of the current cohort.

Depending on data distribution, measurement values were summarized as mean ± standard deviation *(SD)* or median with interquartile range (*IQR*). Statistical comparisons between breeds and between age groups (81 vs. 150 days) were performed using appropriate statistical tests *(*Kruskal-Wallis or *Median test),* based on the normality of the data and the homogeneity of the variances.

Spearman correlation analysis was performed for the total entire experimental cohort, as several variables did not follow a normal distribution. To evaluate the potential influence of haematological and biochemical parameters on feed efficiency traits, multivariate regression analysis was conducted, assessing both overall model significance and the individual contribution of each predictor. Statistical significance was set at $P < 0.05$ for all analyses. Data processing and statistical analyses were performed with SPSS Statistics, version 25 (IBM Corp., 2017).

RESULTS AND DISCUSSION

Description of haematological and biochemical parameters

Haematological and biochemical parameters including IGF-1, insulin, T4, ACTH, haematocrit (HCT), haemoglobin (Hb), and glucose were measured in lambs at the onset of the fattening period, at an average age of 81 days, shortly after weaning. Corresponding data for these parameters following the intensive fattening period, at approximately 150 days of age, have been previously reported (Trapina et al., 2023).

Previous studies have shown that haematological parameters, particularly haematocrit and haemoglobin, exhibit relatively low temporal variability (Ullrey et al., 1965). In contrast, biochemical markers such as IGF-1, insulin, TT4, ACTH, and glucose are more sensitive to dietary composition and feeding conditions (Mahgoub et al., 2015).

Insulin-like growth factor-1

IGF-1, or insulin-like growth factor-1, is a growth hormone–dependent peptide with a short biological half-life of approximately four minutes in sheep (Bruce et al., 1991). IGF-1 plays a central role in development (O'Sullivan et al., 2002) and regulates numerous physiological processes, including cell proliferation, embryogenesis, tissue repair, and the metabolism of carbohydrates, proteins, and lipids. It contributes to both prenatal and postnatal skeletal muscle and bone growth, lipolysis, and mammary gland development (Flores-Encinas et al., 2021). Although the precise role of circulating IGF-1 in determining growth potential remains unclear, its concentration has been shown to correlate with growth rate in sheep (O'Sullivan et al., 2002).

At approximately 81 days of age, the median IGF-1 concentration in lambs was 122.00 (*IQR* 54.00) ng mL^{-1} (Fig. 1). Reference values for IGF-1 in lambs of this age are not reported in the existing literature or laboratory guidelines.

Among breeds, the lowest median IGF-1 levels were observed in TEX (76.50 ng mL^{-1}) and CH (99.00 ng mL^{-1}) lambs, while the highest levels were recorded in IF lambs (147.00 ng mL^{-1}). Notably, the relative breed ranking for IGF-1 concentration remained consistent across both age groups.

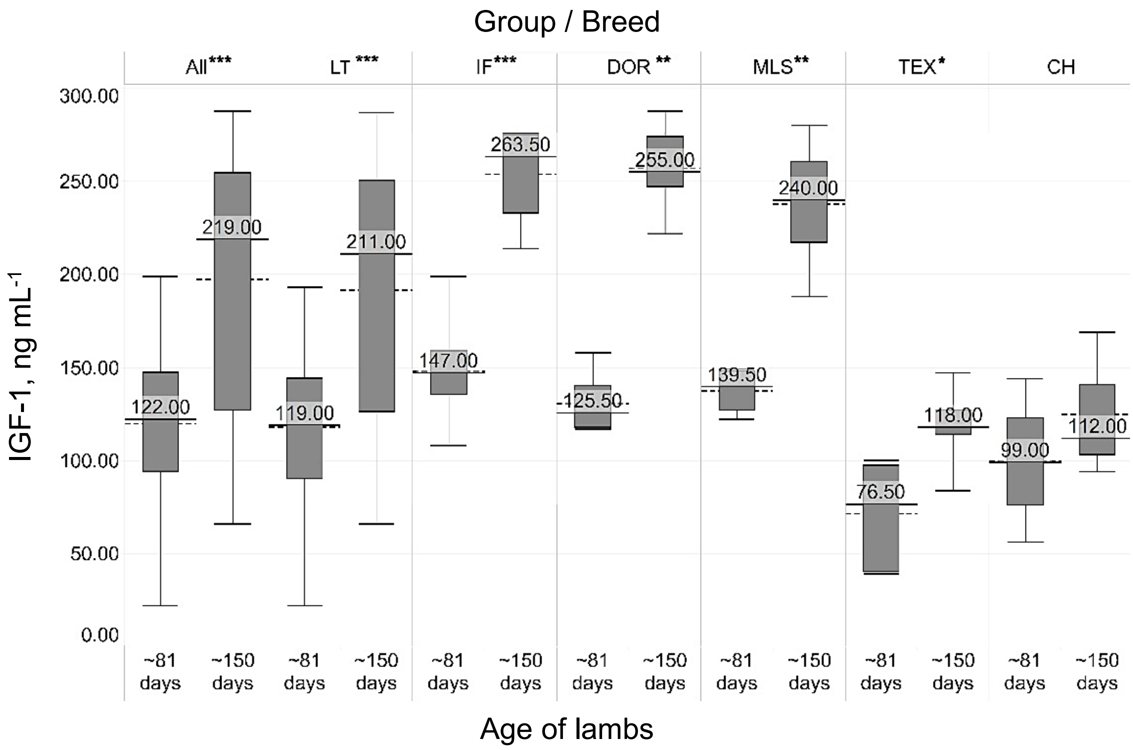

Figure 1. Insulin-like growth factor-1 (IGF-1), ng mL^{-1}, median value (line on box plot) difference for lambs of all/different breeds at age around 81 days (before fattening) and around 150 days (after fattening).

The dashed line on box plot – mean. Breeds of sheep: LT – Latvian dark-head; IF – Île de France; DOR – Dorper; MLS – Merinolandschaf; TEX – Texel; CH – Charollais. *P* value: * < 0.05; ** < 0.01; *** < 0.001.

The IGF-1 concentrations in IF and MLS lambs were significantly higher than those observed in the other Latvian breeds ($P = 2.02 \times 10^{-3}$) (Fig. 1). A similar breed-specific pattern of elevated IGF-1 levels in IF and MLS lambs was also reported in the post-fattening analysis (Trapina et al., 2023).

The overall median IGF-1 concentration at approximately 81 days of age was significantly lower than that observed after fattening, at around 150 days ($P = 6.13\times10^{-11}$). When comparing IGF-1 levels within individual breeds (Fig. 1), statistically significant differences between the pre- and post-fattening measurements were found in all breeds except Charollais (CH), where no significant change was detected.

Notably, the average IGF-1 concentration reported in the serum of healthy Awassi sheep aged 6 to 12 months was approximately 150 ng mL^{-1} (Tarazi et al., 2014), a level comparable to that observed in IF lamb at just 81 days of age in the present study.

Additionally, findings by Flores-Encinas and colleagues (Flores-Encinas et al., 2021) with Dorper x Pelibuey crossbreed sheep, demonstrated an age-dependent increase in IGF-1 levels, consistent with our results, which showed elevated IGF-1 concentrations in older lambs across all breeds.

In contrast to our findings, IGF-1 concentrations in Dorper x Charolais crossbred sheep were reported to decline from 106.2 ng mL^{-1} on day 160 to 58.5 ng mL^{-1} on day 180 (Molina et al., 2021). The discrepancy may be attributed to breed-specific differences, as the animals in that study were crossbreeds, for which our data also revealed considerable variability in IGF-1 levels.

The results indicate that IGF-1 concentrations vary with both age and breed, reflecting genetic diversity under the uniform feeding conditions. However, the current study design does not permit conclusions regarding whether IGF-1 levels increase similarly with age under identical nutritional regimes. Nevertheless, analysis of breed-specific data reveals clear differences in mean IGF-1 concentrations between age groups within the same breed.

Insulin and glucose

Insulin and glucose are key regulators of energy metabolism, with insulin facilitating the cellular uptake and conversion of glucose into usable energy. (Norton et al., 2022). While no established reference range exists for insulin concentrations in sheep, the normal blood glucose range has been reported as 2.78–4.44 mmol L^{-1} (Dias et al., 2010).

At approximately 81 days of age, the median insulin concentration in was 0.40 (*IQR* 0.20) mU L^{-1} (Fig. 2), while the median glucose level was 4.54 (*IQR* 0.63) mmol L^{-1} (Fig. 3) exceeding the reported normal range. No statistically significant differences in insulin levels were observed between breeds; however, glucose concentrations were found to be significantly different ($P = 2.09\times10^{-4}$).

Insulin and glucose levels measured prior to fattening, shortly after weaning, differed significantly from those observed at approximately 150 days of age. Insulin concentrations were significantly lower before fattening ($P = 5.13\times10^{-8}$), whereas glucose levels were significantly higher in the pre-fattening group ($P = 6.16\times10^{-7}$).

In LT, IF and TEX lambs, insulin concentrations at approximately 81 days of age differed significantly from those measured in the post-fattening group with an average age of 151 days (Fig. 2). For the remaining breeds, the difference between age groups were not statistically significant.

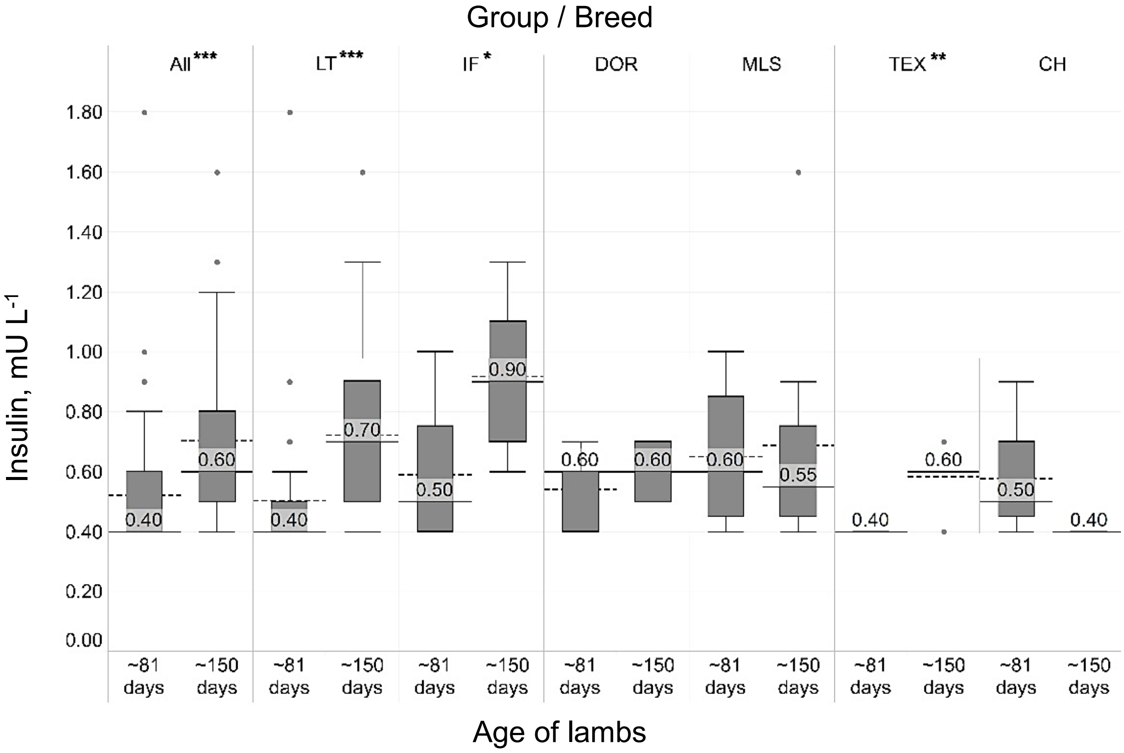

Figure 2. Insulin, mU L^{-1}, mean value difference for lambs of all/different breeds at age average 81 days (~81 days or before fattening) and average 150 days (~150 days or after fattening).

The dashed line on the box plot – mean. Breeds of sheep: LT – Latvian dark-head; IF – Île de France; DOR – Dorper; MLS – Merinolandschaf; TEX – Texel; CH – Charollais. *P* value: * < 0.05; ** < 0.01; *** < 0.001.

The highest glucose concentration at approximately 81 days of age was observed in MLS lambs with a median value of 5.07 mmol L^{-1} (*IQR* 0.24) (Fig. 3). Notably, all MLS lambs in this age group exhibited glucose levels exceeding the laboratory-determined normal range.

All DOR lambs at approximately 81 days of age also exhibited glucose concentrations above the established normal range. However, their levels were nearly 0.5 units lower than those of MLS lambs, despite identical feeding conditions during the adaptation period. Similarly, at least half of the lambs in the LT, IF and CH breeds showed elevated glucose levels. TEX was the only breed in which nearly all lambs had glucose concentrations within normal range.

Significant differences in glucose concentrations between pre- and post-fattening stages were observed in LT and MLS breeds. In both breeds, glucose levels were lower following fattening and, on average, fell within the normal reference range, in contrast to elevated levels observed prior to fattening.

IF breed was the only breed in which glucose concentrations increased at 150 days of age compared to levels at approximately 81 days.

The results obtained in this study are not directly comparable to those of previous research due to differences in housing conditions, feeding regimens, and animal age. For instance, in Corriedale x Suffolk sheep aged 2–4 years, insulin concentrations ranged from 10 till 16 mU L^{-1} under different dietary conditions (Sano et al., 2003). In contrast, insulin levels in blue-faced Leicester cross Swaledale sheep at 11 months of age were reported to range from 0.14 to 0.16 mU L^{-1} (Gardner et al., 2005), which is more consistent with the values observed in both age groups of our study.

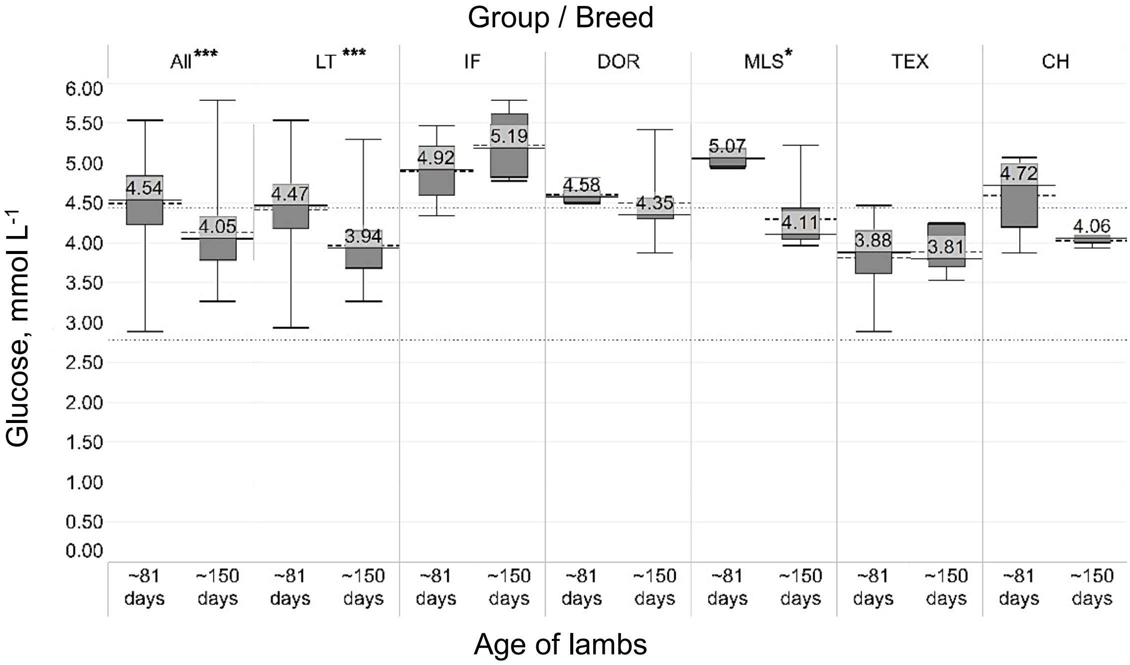

Figure 3. Glucose, mmol L^{-1}, mean value difference for lambs of all/different breeds average 81 days (~81 days or before fattening) and 150 days (~150 days or after fattening).

The dashed line on the box plot – mean. Breeds of sheep: LT – Latvian dark-head; IF – Île de France; DOR – Dorper; MLS – Merinolandschaf; TEX – Texel; CH – Charollais. *P* value: * < 0.05; ** < 0.01; *** < 0.001. Dotted line – range of normal level: 2.78 – 4.44 mmol L^{-1} (Dias et al., 2010).

In contrast to insulin, glucose concentrations have been more frequently reported in the literature with minimal methodological variation. For example, in Rahmani male lambs at 162 days of age, glucose levels were measured at 54.0 ± 1.49 mg dL^{-1} (equivalent to 3.0 ± 0.1 mmol L^{-1}) (Nour El-Din et al., 2009). In Dalmatian Pramenka sheep aged four to six months, glucose concentrations averaged 4.80 ± 0.10 mM (Vojta et al., 2011). For adult Portuguese Churra-da-Terra-Quente ewes, aged two to seven years and weighing approximately 55 kg, glucose levels were 2.87 ± 0.60 mmol L^{-1} (Dias et al., 2010).

The observed variability in insulin and glucose levels across studies and breeds supports the conclusion by Vojta et al. (2011) that breed-specific reference values are necessary. Findings from the present study also suggest that these normative values may also vary with age.

Thyroxine

Thyroid hormones, primarily thyroxine (T4) which accounts for approximately 93% of circulating thyroid hormone, regulate nuclear transcription in nearly all cell types and influence overall physiological activity (Sampaio et al., 2021). The reference range for total T4 is 38.6–77.2 nmol L^{-1} (Dias et al., 2010).

A reduced metabolic rate of thyroid hormones can result to hypothermia and cold intolerance (Tsibulnikov et al., 2020). Thyroxine also regulates key metabolic processes involved in maintaining the body's energy balance, including the metabolism of fats, proteins, vitamins and other nutrients (Duru et al., 2019; Flis & Molik, 2021). T4 are known to change in response to physiological stress, illness (including parasitic infections), and environmental change (Duru et al., 2019; Flis & Molik, 2021; Sampaio et al., 2021; Maiden et al., 2024). Therefore, maintaining stable T4 concentrations during feeding may indicate that the animal is in a physiological comfort zone.

For all samples of 81-day-old lambs of sheep breeds raised in Latvia, the median T4 is 79.00 (*IQR* 31.75) nmol L^{-1} (Fig. 4). This overall median exceeds the established reference range, and statistically significant differences were observed between breeds ($P = 5.22 \times 10^{-3}$). The highest median T4 level before fattening was recorded in MLS breed lambs (135.50 nmol L^{-1}), while the lowest values were observed in TEX and CH lambs (66.50 nmol L^{-1} for both). Medians of T4 concentrations in all breeds were either above or near the upper limit of the normal reference range.

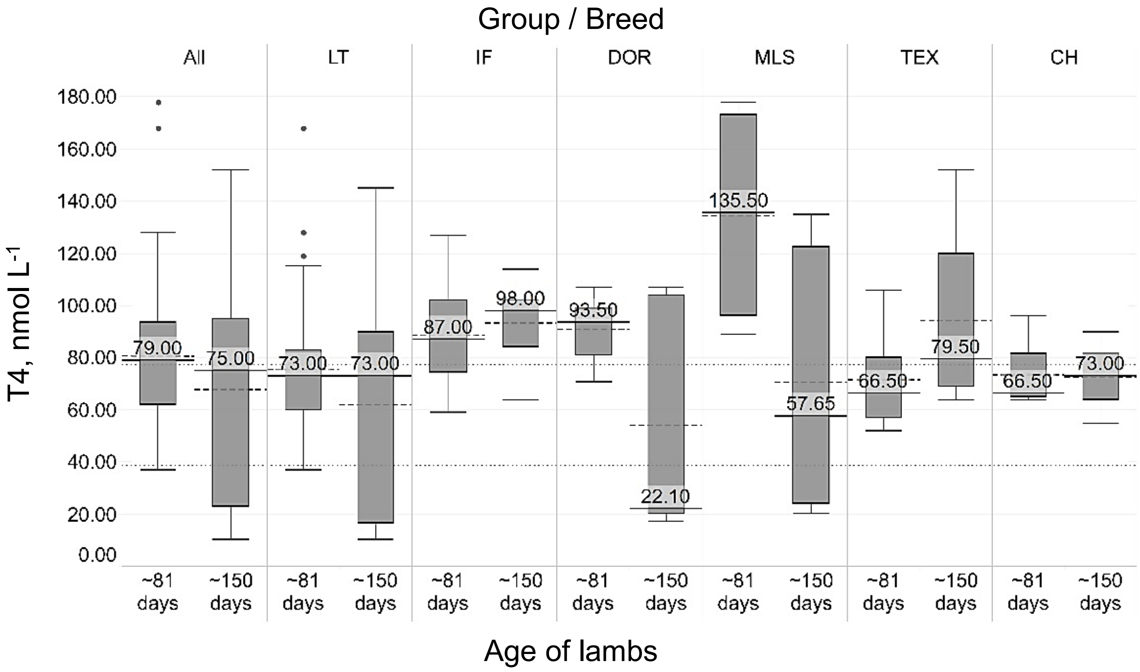

Figure 4. Thyroxine (T4), nmol L^{-1}, mean value difference for lambs of all/different breeds in age average 81 days (~81 days or before fattening) and average 150 days (~150 days or after fattening).

The dashed line on the box plot – mean. Breeds of sheep: LT – Latvian dark-head; IF – Île de France; DOR – Dorper; MLS – Merinolandschaf; TEX – Texel; CH – Charollais. *P* value: * < 0.05; ** < 0.01; *** < 0.001. Dotted line – range of normal level: 38.6–77.2 nmol L^{-1} (Dias et al., 2010).

Comparison of T4 levels before (81 days of age) and after fattening (150 days of age), revealed no statistically significant differences. Although a broader range of T4 values was observed in certain breeds, specifically for LT, DOR, MLS and TEX, the median values remained consistent overall.

Previous studies have reported varying average T4 concentrations in healthy sheep, depending on breed, age, and physiological status. In three- to five-year-old Santa Inês and crossbred Dorper × Santa Inês T4 levels ranged from 40.55 to 45.08 nmol L^{-1} (Sampaio et al., 2021). In Karayaka sheep with an average body weight of 55.64 ± 4.66 kg prior to slaughter, T4 levels were reported as 7.46 ± 1.07 µg dL^{-1} equivalent to 96.01 ± 13.77 nmol L^{-1}(Tozlu Çelik et al., 2021). Iranian fat-tailed sheep aged 1 to 2 years showed T4 concentrations of 49.46 nmol L^{-1} (Ekiz et al., 2013), while the highest values were observed in Akkaraman sheep (8.52 ± 0.23 µg dL^{-1} or 109.65 ± 2.96 nmol L^{-1}) (Duru et al., 2019).

Comparison of the present findings with previously published studies indicates that normal T4 levels in healthy animals vary considerably between breeds. Based on available data, the physiological T4 range in sheep may span from approximately 40 to 140 nmol L^{-1}, or potentially even broader, depending on breed-specific and individual factors.

Changes in thyroid hormone levels contribute to the regulation of metabolic balance and adaptation to environmental conditions in animals (Tozlu Çelik et al., 2021). Therefore, the relatively stable T4 concentrations observed before and after intensive feeding, across different age stages, may indicate that the controlled fattening environment provided favourable conditions for the lambs.

Adrenocorticotropic hormone

Plasma concentrations of adrenocorticotropic hormone (ACTH) vary in response to the animal's physiological state, as ACTH plays a central role in maintaining homeostasis and mediating responses to environmental challenges (Amokrane-Ferrah et al., 2022). ACTH acts on the adrenal cortex axis, a key component of the neuroendocrine stress response and, works in concert with behavioural adaptations, to support overall physiological adaptation (Mormede & Terenina, 2012).

ACTH secretion is rhythmic in most species, occurring approximately every 90 minutes, and is influenced by circadian cycle, feeding, physical activity, and environmental conditions (Mormède et al., 2007). The reported reference range for ACTH in plasma is 50–100 pg mL^{-1} (Amokrane-Ferrah et al., 2022).

For all samples of 81-day-old lambs of sheep breeds raised in Latvia, the median ACTH concentration was 46.15 pg mL^{-1} (*IQR* 45,93) (Fig. 5), which falls below the established reference range. Although some individuals exhibited markedly elevated ACTH levels, no statistically significant differences were observed between breeds. This lack of significance is probably due to the high variability within breed groups.

Among all samples, the lowest ACTH concentration was 11.50 pg mL^{-1}, observed in the CH lamb, while the highest was 549.00 pg mL^{-1} in the IF lamb. All breeds, except CH, included individuals with ACTH values beyond the limits of the normal reference range. Approximately 5% of all samples exhibited ACTH concentrations above or below the normal limits, aligning with the expected proportion of values outside reference intervals in a healthy population (Seixas et al., 2021).

As ACTH levels are known to rise under stressful conditions (Mormède et al., 2007), elevated values above the normal range may be related to the blood sampling procedure. However, this does not account for the occurrence of unusually low ACTH concentrations.

Following intensive fattening, ACTH levels continued to exhibit wide variability (Trapina et al., 2023). No statistically significant differences were observed between the pre- and post-fattening groups ($P > 0.05$). Breed-specific trends were noted: ACTH levels were lower at 81 days of age in LT, DOR, TEX and CH lambs, while higher concentrations were observed in young IF and MLS lambs.

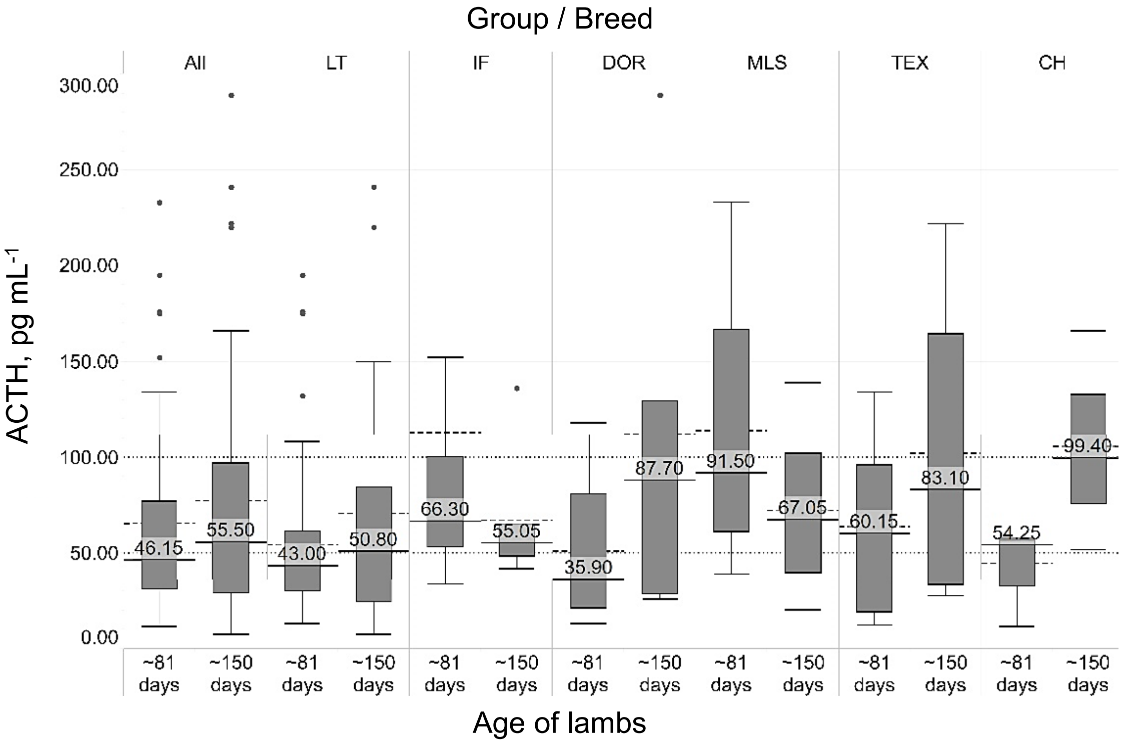

Figure 5. Adrenocorticotropic hormone (ACTH), pg mL^{-1}, mean value difference for lambs of all/different breeds in age average 81 days (~81 days or before fattening) and average 150 days (~150 days or after fattening).

The dashed line on the box plot – mean. Breeds of sheep: LT – Latvian dark-head; IF – Île de France; DOR – Dorper; MLS – Merinolandschaf; TEX – Texel; CH – Charollais. *P* value: * < 0.05; ** < 0.01; *** < 0.001. Dotted line – range of normal level: 50–100 pg mL^{-1} (Amokrane-Ferrah et al., 2022).

The observed differences in median ACTH levels between breeds may be partially explained by variation in the timing of fattening and associated weather conditions, despite all animals being housed indoors under standardized conditions within a single year. Seasonal variation in ACTH concentrations has been reported in two-year-old Ouled Djellal and D'Man sheep breeds, where levels ranged from 10 to 100 pg mL^{-1} depending on the season, being lower in winter and higher in summer (Amokrane-Ferrah et al., 2022).

Haematocrit and haemoglobin

Haematocrit (HCT) reflects the proportion of red blood cells in the total blood volume, while haemoglobin (Hb) is an iron-containing protein within red blood cells responsible for transporting oxygen to body tissues (Fanta et al., 2024). Adequate haemoglobin levels are essential to maintain effective tissue oxygenation. The reference range for HCT is 27.0–45.0% (Dias et al., 2010), and for Hb, 90.00–150 g L^{-1} (Latimer 2011).

At 81 days of age, haematocrit median values were below the reference range in all analysed breeds, with an overall median of 21.00% (*IQR* 6.00) (Fig. 6). Breed-specific median HCT values ranged from 20.00% in MLS lambs to 23.50% in CH lambs, with no statistically significant differences between breeds ($P = 0.78$).

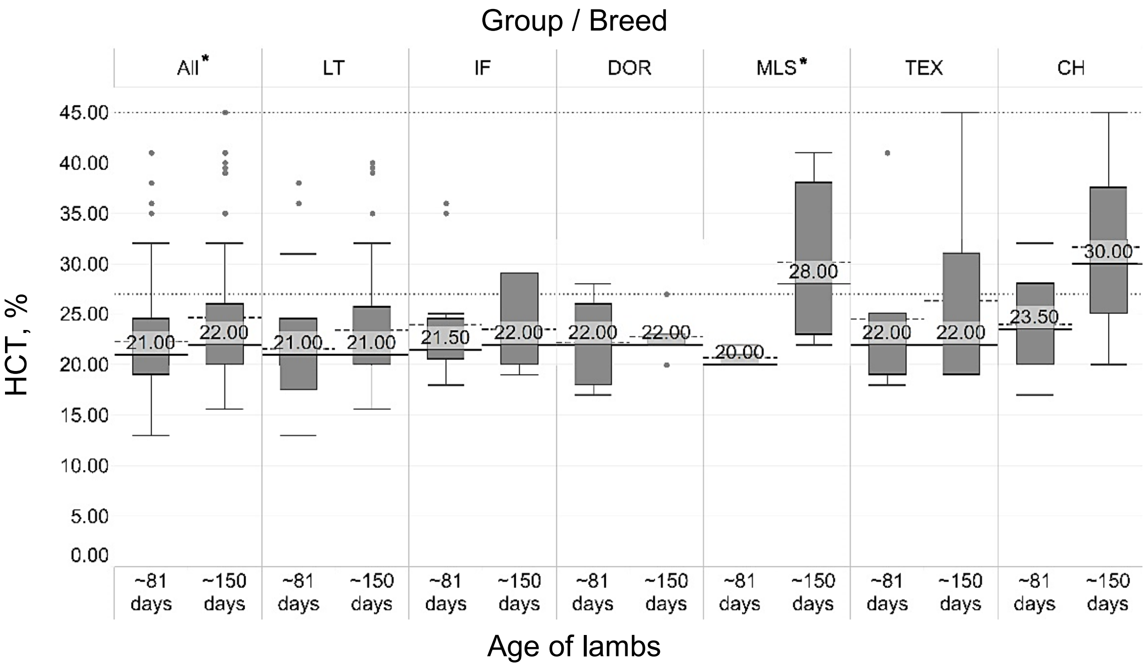

Figure 6. Haematocrit (HCT), %, mean value difference for lambs of all/different breeds in age average 81 days (~81 days or before fattening) and average 150 days (~150 days or after fattening).

The dashed line on the box plot – mean. Breeds of sheep: LT – Latvian dark-head; IF – Île de France; DOR – Dorper; MLS – Merinolandschaf, TEX – Texel; CH – Charollais. *P* value: * < 0.05; ** < 0.01; *** < 0.001. Dotted line – range of normal level: 27.0–45.0% (Dias et al., 2010).

Comparing HCT values before and after fattening revealed a statistically significant difference across all lambs ($P = 2.07\times10^{-2}$). This effect was primarily driven by the MLS breed, where HCT levels increased significantly from a median of 20.00% (*IQR* 0.00) at 81 days of age to 28.00% (*IQR* 16.50) at 150 days ($P = 1.78\times10^{-2}$).

In both the MLS and CH breed groups, HCT levels were within the normal reference range after fattening. In the remaining breeds, the HTC level either remained unchanged or showed a slight increase, though still below the lower limit of the reference range.

HCT values below the normal range have been reported in various species. For example, in Ethiopian Doyogena sheep, HCT levels ranged from 9.57 to 11.19%, depending on dietary conditions (Fanta et al., 2024). In sheep, HCT levels below 24.00% are generally considered indicative of anaemia risk (Seixas et al., 2021). However, all lambs in our study were under routine veterinary supervision and exhibited no clinical signs of illness.

In contrast, haematocrit levels in various sheep breeds in Brazil have been reported to range from 28.1 to 31.5%, with significant variation observed across age groups (Seixas et al., 2021). Similarly, in four- to six-month-old Dalmatian pramenka sheep, HTC values fell within the normal range, averaging 31.73 ± 0.37% (Vojta et al., 2011).

A study (Barsila et al., 2020) investigating the effect of altitude and vegetation on HCT levels in Baruwal sheep reported values ranging from 21.18% in year-old sheep at low altitudes to 40.63% at high altitudes. Furthermore, HCT levels at low altitudes were consistently below the reference range regardless of sex or age.

Summarising the results, it can be concluded that reduced HCT levels do not necessarily indicate pathology, as breed-specific physiological norms may differ. In the Latvian sheep population, HCT values did not change significantly with age or intensive fattening in most breeds, with the exception of MLS, where a significant increase was observed.

At 81 days of age, median haemoglobin concentration across all lamb breeds was 121.00 g l^{-1} (*IQR* 14.00) (Fig. 7). Hb levels in nearly all lambs fell within the established normal range. A statistically significant difference in Hb concentrations was observed between breeds at this age ($P = 3.00 \times 10^{-2}$). The lowest median Hb level was recorded for the MLS breed: 111.00 g L^{-1}, and the highest was observed in the TEX breed (129.50 g L^{-1}).

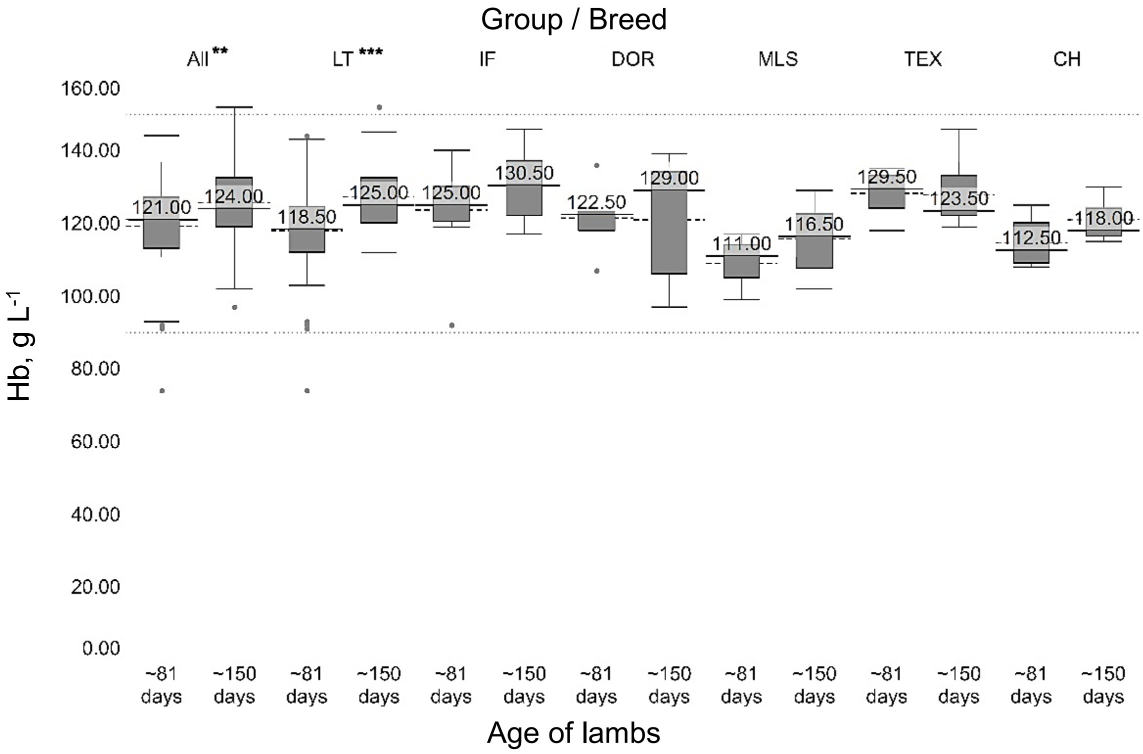

Figure 7. Haemoglobin (Hb), g L^{-1}, mean value difference for lambs of all/different breeds in age average 81 days (~81 days or before fattening) and average 150 days (~150 days or after fattening).

The dashed line on the box plot – mean. Breeds of sheep: LT – Latvian dark-head, IF – Île de France, DOR – Dorper; MLS – Merinolandschaf; TEX – Texel; CH – Charollais. *P* value: * < 0.05; ** < 0.01; *** < 0.001. Dotted line – range of normal level: 90.0 – 150.0 g L^{-1} (Latimer 2011).

A comparison of Hb levels in Latvian sheep breeds before fattening (81 days of age) and after fattening (150 days of age) revealed a statistically significant difference only in the LT group ($P = 3.62 \times 10^{-4}$), where younger lambs had lower Hb levels (118.50 g L^{-1}) compared to older lambs (125.00 g L^{-1}). A similar pattern, lower Hb levels before fattening and higher levels after, was observed in most other breeds, with the exception of TEX, in which post-fattening Hb levels were slightly reduced (129.00 vs 123.50 g L^{-1}) and showed greater variability.

Similar to the case of HCT, Hb levels also vary across breeds, not only within the Latvian sheep population but globally. In Rahmani male lambs at 162 days of age, Hb concentrations were reported at 104.5 ± 1.8 g L^{-1} (Nour El-Din et al., 2009), while in Dalmatian pramenka sheep aged four to six months, values averaged 102 ± 1.20 g L^{-1} (Vojta et al., 2011). In Ethiopian Doyogena sheep, Hb concentrations ranged from 89.6, below the normal reference range, to 105.8 g L^{-1}, depending on diet (Fanta et al., 2024). In Brazilian sheep breeds, Hb values ranged from 81.3 to 97.0 g L^{-1}, with significant differences observed between adult and young animals (Seixas et al., 2021).

Relationships between parameters

As part of ram evaluation within breeding programs, lambs in this study underwent intensive fattening to assess growth performance. Growth traits were recorded for each animal and used to calculate multiple feed efficiency indicators including feed efficiency (FE), feed conversion ratio (FCR), relative growth rate (RGR), and Kleiber's ratio (KR), as well as residual-based metrics such as residual feed intake (RFI), residual weight gain (RWG), and residual intake and body weight gain (RIG) (Berry & Crowley, 2012; Lima et al., 2017). These indicators were calculated at approximately 150 days of age. A previous study (Trapina et al., 2023) examined the associations between haematological and biochemical parameters at 150 days and feed efficiency traits, along with interrelationships among the blood parameters themselves.

This study investigated the relationship between haematological and biochemical parameters determined at an average age of 81 days and feed digestion parameters assessed after fattening, at approximately 150 days of age. Correlation analysis was performed on the entire experimental cohort to evaluate associations between early-life blood parameters and subsequent feed efficiency indicators (Fig. 8).

The results indicate that IGF-1 and glucose concentrations measured at 81 days of age are significantly correlated with feed efficiency indicators, specifically FE/FCR, RGR and KR, after fattening. For IGF-1, the correlation with these indicators remains significant even when measured post-fattening. In contrast, glucose levels at 150 days of age were significantly associated only with RGR and KR, while pre-fattening glucose levels showed broader associations. Notably, post-fattening glucose concentrations were also correlated with with RWG and RFI, relationships not observed for glucose values measured prior to fattening.

Following fattening, ACTH concentrations showed a significant correlation with RFI. Although the same correlation coefficient was observed for ACTH measured at 81 days of age, the level of statistical significance was reduced. Similar results were previously reported in crossbred rams, where residual feed intake was directly proportional to cortisol release following ACTH injection (Knott et al., 2008).

-1.0 0.0 1.0

	FE	FCR	RGR	KR	RWG	RFI	RIG
IGF-1	**0.37**	**-0.37**	**0.55**	**0.63**	**0.25**	0.07	0.11
Insulin	0.15	-0.16	**0.31**	**0.33**	0.16	0.14	0.01
Glucose	**0.34**	**-0.34**	**0.30**	**0.42**	0.11	-0.19	<u>0.20</u>
T4	0.00	0.00	<u>-0.22</u>	-0.17	-0.11	-0.14	0.05
ACTH	0.19	-0.19	-0.08	-0.03	0.09	<u>-0.22</u>	0.19
HCT	0.08	-0.08	-0.03	-0.02	0.08	-0.16	0.12
Hb	0.14	-0.14	<u>0.21</u>	<u>0.19</u>	<u>0.20</u>	0.10	0.07

	FE	FCR	RGR	KR	RWG	RFI	RIG
IGF-1	**0.45**	**-0.48**	**0.55**	**0.63**	**0.44**	0.10	0.20
Insulin	0.14	-0.16	0.14	0.14	0.08	0.00	0.05
Glucose	0.10	-0.11	**0.23**	**0.35**	**0.23**	**0.23**	0.00
T4	-0.05	0.02	-0.18	-0.14	-0.10	-0.10	0.00
ACTH	0.07	0.03	-0.09	-0.09	**0.06**	**-0.22**	0.16
HCT	0.06	-0.01	-0.05	-0.04	-0.04	-0.07	0.02
Hb	0.20	**-0.22**	0.19	0.19	0.12	-0.01	0.07

Figure 8. Correlation of haematological/biochemical parameters (measured at age 81 (upper square) and age 150 (lower square; Trapina et al., 2023)) and feed efficiency indicators (calculated at lambs' age around 150 days) for lambs of all breeds.

IGF-1 – Insulin-like growth factor-1; T4 – Thyroxine; ACTH – Adrenocorticotropic hormone; HCT – haematocrit; Hb – haemoglobin; FCR – Feed conversion ratio; FE – Feed efficiency; RGR – Relative growth rate; KR – Kleiber ratio; RFI – Residual feed intake. RWG – Residual weight gain; RIG – Residual intake and body weight gain. Value with statistical significance $P < 0.05$ is highlighted in bold, and P = [0.05–0.09] is underlined.

Additionally, studies in Hu lambs demonstrated that animals with lower RFI values exhibited reduced levels of TT4 (total Thyroxine) and ACTH, with a positive association between these hormones; also, a positive correlation between FCR and TT4 (Zhang et al., 2017). In contrast, our results indicate that in Latvian sheep breeds, T4 levels do not significantly correlate with feed efficiency indicators. This observation aligns with findings from Ramos et al. (2021), who reported no significant effect of RFI score level on biochemical and haematological parameters in the Dorper breed.

Multivariate regression analysis was performed to determine whether pre-fattening haematological and biochemical measurements could serve as predictive markers of feed efficiency. The regression analysis (Table 1) determined that these parameters statistically significantly

Table 1. Haematological and biochemical parameters before fattening significant ($P < 0.05$) regression to feed efficiency indicators after a fattening period in sheep breeds raised in Latvia

Regression model variables/ indicators	Feed efficiency indicators /		
	FCR	RGR	KR
R^2	0.25	0.23	0.32
Regression model coefficients Ɓ for the equation			
Constant	7.59	0.063	0.18
IGF-1	-4.71 x10^{-5}	4.74 x10^{-4}	0.023
Insulin	-0.036	-0.056	-2.66
Glucose	-0.40	0.054	2.58*
T4	-0.008	1.78 x10^{-6}	0.008
ACTH	-0.002	1.99 x10^{-4}	0.009
HCT	0.022	-0.002	-0.020
Hb	-0.003	0.001	0.050

R^2 – the coefficient of determination; IGF-1 – Insulin-like growth factor-1; T4 – Thyroxine; ACTH – Adrenocorticotropic hormone; HCT – haematocrit; Hb – haemoglobin; FCR – Feed conversion ratio; RGR –Relative growth rate; KR – Kleiber ratio. * $P < 0.05$.

explained the variation in three key feed efficiency parameters following intensive fattening: FCR ($P = 4.57\times10^{-3}$), RGR ($P = 9.04\times10^{-3}$) and KR ($P = 2.75\times10^{-4}$).

When combining all seven biochemistry and haematology parameters measured at 81 days of age, it is possible to explain 25% of the variance in FCR, 23% in RGR and 32% in KR at 150 days of age. These findings suggest the potential to predict feed digestion efficiency in lambs based on early physiological indicators.

CONCLUSIONS

In the Latvian sheep population, median glucose and T4 levels at approximately 81 days of age exceeded established normal reference ranges, whereas the ACTH and haematocrit levels were below those thresholds. The results of this study also demonstrate significant breed-specific differences in IGF-1, glucose, T4, and haemoglobin concentrations among Latvian dark-head, Merinolandschaf, Île de France, Charollais, Dorper and Texel lambs at this developmental stage.

IGF-1, Insulin, Glucose, Haematocrit and Haemoglobin levels in the Latvian sheep population differ significantly between the pre- and post-fattening periods.

The observed correlations and significant regression models between the blood biochemical parameters and lambs' feed efficiency indicators show the possibility of hormonal influence on feed efficiency in Latvian sheep breeds.

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