

# CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

Leila Bennani Ziatni



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**CLIMATE CHANGE IMPACTS ON AGRICULTURE  
AND ANIMAL NUTRITION- 2026**

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# **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

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## TABLE OF CONTENTS

<b>PREFACE.....</b>	i
<b>CHAPTER 1</b>	
<b>PROJECTED TEMPERATURE CHANGES UNDER SSP</b>	
<b>SCENARIOS AND THEIR IMPLICATIONS FOR</b>	
<b>AGRICULTURE IN ARID ZONES: A CASE STUDY OF</b>	
<b>BISKRA, ALGERIA</b>	
Ameur ZAGHOUANI.....	1
<b>CHAPTER 2</b>	
<b>WHEY AS AN ADDITION IN THE DIET AND ITS EFFECT ON</b>	
<b>THE LIVER</b>	
Kristina TOMSKA	
Julijana TOMOVSKA.....	32
<b>CHAPTER 3</b>	
<b>MONOGASTRIC NUTRITION AND WATER IN ANIMAL</b>	
<b>NUTRITION</b>	
Emmanuel Olumide AWOBONA.....	79

## PREFACE

This book brings together interdisciplinary studies that explore the interactions between climate change, agriculture, and animal nutrition in environmentally sensitive regions. The chapters collectively emphasize the need for science-based strategies to ensure sustainable food production, resource efficiency, and resilience under changing climatic and nutritional conditions.

The chapter Projected Temperature Changes under SSP Scenarios and Their Implications for Agriculture in Arid Zones: A Case Study of Biskra, Algeria examines future climate risks using scenario-based projections, highlighting potential impacts on agricultural productivity in arid environments. This environmental perspective is complemented by Whey as an Addition in the Diet and Its Effect on the Liver, which investigates the nutritional and physiological effects of dietary supplementation, contributing to improved animal health and feed utilization.

The final chapter, Monogastric Nutrition and Water in Animal Nutrition, focuses on the critical relationship between feed composition and water use in animal production systems. Together, these chapters provide a concise yet integrated view of how climate analysis and nutritional science can inform sustainable agricultural and livestock management practices, offering valuable insights for researchers, practitioners, and policymakers alike.

**January 26, 2025**  
**Türkiye**

*CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL  
NUTRITION*

**CHAPTER 1**  
**PROJECTED TEMPERATURE CHANGES UNDER**  
**SSP SCENARIOS AND THEIR IMPLICATIONS FOR**  
**AGRICULTURE IN ARID ZONES: A CASE STUDY OF**  
**BISKRA, ALGERIA**

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# ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

## **INTRODUCTION**

Climate change has emerged as one of the most critical challenges facing environmental engineers in the twenty-first century, particularly in arid and semi-arid regions where natural systems already operate close to their ecological and hydrological limits (Abdullaeva, 2024). Rising air temperatures, increased frequency of extreme heat events, and shifts in thermal regimes are profoundly altering water availability, ecosystem functioning, agricultural productivity, and the performance of environmental infrastructure. In this context, temperature is not merely a climatic variable but a key driver that conditions the sustainability and resilience of both natural and engineered systems (Abdullaeva, 2024; Lutta et al., 2024).

Arid regions are especially vulnerable to temperature increases due to their limited precipitation, high evapotranspiration rates, and strong dependence on climate-sensitive sectors (Liu et al., 2024; Xu et al., 2025). Even modest warming can translate into disproportionate environmental impacts, including accelerated water scarcity, soil degradation, salinization, and increased stress on agricultural and urban systems (Gonçalves et al., 2017; Salam, 2019). From an environmental engineering perspective, understanding present and future temperature dynamics is therefore essential for designing adaptive water management strategies, resilient agricultural systems, and climate-responsive urban environments (Abdennour et al., 2020; Boudiaf et al., 2020).

The Biskra region, located in southeastern Algeria, represents a representative case study of arid environments in North Africa. Characterized by extremely hot summers, low and highly variable rainfall, and growing anthropogenic pressures, Biskra is already experiencing the combined effects of climate variability and socio-economic development (Boudiaf et al., 2020; Guermazi et al., 2018). The region hosts intensive irrigated agriculture, oasis ecosystems, and expanding urban areas, all of which are highly sensitive to thermal stress. Consequently, anticipating future temperature changes in Biskra is of paramount importance for long-term environmental planning and sustainable development (Hoseini et al., 2024; Martel et al., 2022).

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

Recent advances in climate modeling, particularly within the framework of the Coupled Model Intercomparison Project Phase 6 (CMIP6), have enabled the development of Shared Socioeconomic Pathways (SSPs) that link greenhouse gas emission trajectories with socio-economic development patterns (Hoseini et al., 2024; Salman et al., 2022; Yao et al., 2021). These scenarios provide a robust and coherent basis for assessing future climate conditions under different mitigation and adaptation pathways. For environmental engineers, SSP-based projections offer valuable insights into how alternative development choices may shape future environmental constraints and risks. (Adekanmbi et al., 2023 ; Belay et al., 2025 ; Hoseini et al., 2024 ; Marangoni et al., 2017).

Within this framework, spatially explicit heat maps constitute a powerful analytical tool for visualizing and interpreting projected temperature changes across different time horizons and emission scenarios. By capturing variations in minimum, mean, and maximum surface air temperatures, heat maps allow for a comprehensive assessment of both average conditions and extreme thermal stress (Huang et al., 2019; Murakami et al., 2021; Zhou et al., 2022). Such information is particularly relevant for environmental engineering applications, as minimum temperatures influence nocturnal cooling and ecosystem recovery, mean temperatures define baseline environmental conditions, and maximum temperatures determine the intensity and frequency of heat-related risks (Huang et al., 2021; Li et al., 2020; Sherman et al., 2022).

The objective of this chapter is to analyze and interpret projected changes in surface air temperature in the Biskra region throughout the twenty-first century using heat maps derived from SSP-based climate scenarios. The chapter focuses on average annual minimum, mean, and maximum temperatures across multiple future periods, with particular emphasis on their implications for environmental engineering. Rather than limiting the analysis to a purely climatological perspective, the chapter explicitly links temperature projections to key engineering concerns, including water resource management, agricultural sustainability, urban heat mitigation, and climate-resilient infrastructure design.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

By integrating climate projections with an environmental engineering perspective, this chapter aims to contribute to a deeper understanding of how future thermal conditions may shape the challenges and opportunities of sustainable development in arid regions. The insights provided are intended to support engineers, planners, and decision-makers in developing adaptive strategies that are scientifically informed, regionally relevant, and aligned with long-term climate resilience objectives.

### **1. METHODOLOGICAL FRAMEWORK**

This chapter adopts an integrated methodological framework combining climate scenario analysis, spatial temperature mapping, and environmental engineering interpretation to assess future thermal conditions in the Biskra region. The approach is designed to move beyond a purely climatological description by explicitly linking projected temperature changes to environmental engineering concerns relevant to arid regions.

#### **1.1 Study Area and Environmental Context**

The study focuses on the Biskra region, located in southeastern Algeria, which is characterized by an arid climate with high interannual temperature variability, extremely hot summers, and limited precipitation (Boudiaf et al., 2020). The region is environmentally and socio-economically significant due to the coexistence of oasis ecosystems, irrigated agricultural systems, and rapidly expanding urban areas (Ait-El-Mokhtar et al., 2020). These characteristics make Biskra particularly sensitive to changes in surface air temperature and an appropriate case study for assessing climate change impacts from an environmental engineering perspective (Hoseini et al., 2024).

#### **1.2 Climate Scenarios and Shared Socioeconomic Pathways (SSPs)**

Future temperature projections analyzed in this chapter are based on the Shared Socioeconomic Pathways (SSPs) developed within the framework of the Coupled Model Intercomparison Project Phase 6 (CMIP6) (Abdelmoaty et al., 2025; Hoseini et al., 2024).

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

SSPs provide internally consistent narratives linking socio-economic development, greenhouse gas emissions, and climate outcomes, thereby enabling the exploration of alternative future pathways (Marangoni et al., 2017; Meinshausen et al., 2020).

Four SSP scenarios were considered to capture a broad range of possible futures:

- **SSP1-2.6:** representing a sustainability-oriented pathway with strong mitigation efforts and low greenhouse gas emissions (Marangoni et al., 2017).
- **SSP2-4.5:** reflecting an intermediate pathway characterized by moderate mitigation and continued socio-economic development (Marangoni et al., 2017).
- **SSP3-7.0:** depicting a fragmented world with limited mitigation and high emissions (Marangoni et al., 2017).
- **SSP5-8.5:** representing a fossil-fueled development pathway with very high emissions (Marangoni et al., 2017).

The selection of these scenarios allows for a comparative assessment of low-, intermediate-, and high-emission futures and their implications for environmental systems and engineering design (Pedersen et al., 2020; Scott et al., 2019).

### **1.3 Temporal Horizons and Climate Projection Periods**

To analyze the temporal evolution of temperature changes throughout the twenty-first century, projections were examined across four distinct future periods:

- Near future: 2020–2039
- Mid-century: 2040–2059
- Late-century: 2060–2079
- End of century: 2080–2100

This temporal stratification enables the identification of both gradual trends and acceleration phases in warming, which is essential for distinguishing short-term adaptation needs from long-term strategic planning in environmental engineering.

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

### **1.4 Temperature Indicators and Analytical Variables**

Three complementary temperature indicators were selected to provide a comprehensive characterization of future thermal conditions:

- Average annual minimum surface air temperature: reflecting nighttime thermal conditions and ecosystem recovery potential (Woolway et al., 2019).
- Average annual mean surface air temperature: representing baseline climatic conditions relevant to long-term environmental processes (Woolway et al., 2019).
- Average annual maximum surface air temperature: capturing daytime extremes and heat stress intensity (Woolway et al., 2019).

The combined analysis of these indicators allows for the assessment of both average warming and extreme thermal risks, which are critical for evaluating impacts on water resources, agriculture, urban environments, and infrastructure performance.

### **1.5 Spatial Analysis Using Heat Maps**

Heat maps were employed as the primary spatial analysis tool to visualize and interpret projected temperature changes across the study area. This approach enables the identification of spatial patterns, temporal trends, and scenario-dependent differences in thermal conditions (Lyu et al., 2024; Meenar et al., 2023).

From an environmental engineering standpoint, heat maps provide a valuable means of translating complex climate model outputs into actionable spatial information (Jagarnath et al., 2020). They facilitate the identification of areas exposed to higher thermal stress, support vulnerability assessments, and inform spatially explicit adaptation measures such as targeted irrigation strategies, urban heat mitigation, and land-use planning (Eingrüber et al., 2024; Lau et al., 2025).

### **1.6 Analytical Approach and Scenario Comparison**

The methodological approach combines qualitative and comparative analysis of heat maps across scenarios and time periods. Emphasis is placed on identifying:

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

- The magnitude and direction of temperature changes.
- The divergence between SSP scenarios over time.
- Critical thresholds and acceleration point relevant to environmental systems.

Scenario comparisons are used to highlight the influence of socio-economic pathways and mitigation efforts on future thermal conditions, thereby providing a basis for risk-based environmental planning and decision-making.

### **1.7 Environmental Engineering Interpretation Framework**

To ensure relevance to environmental engineering, projected temperature changes are interpreted through their implications for key engineered and natural systems, including:

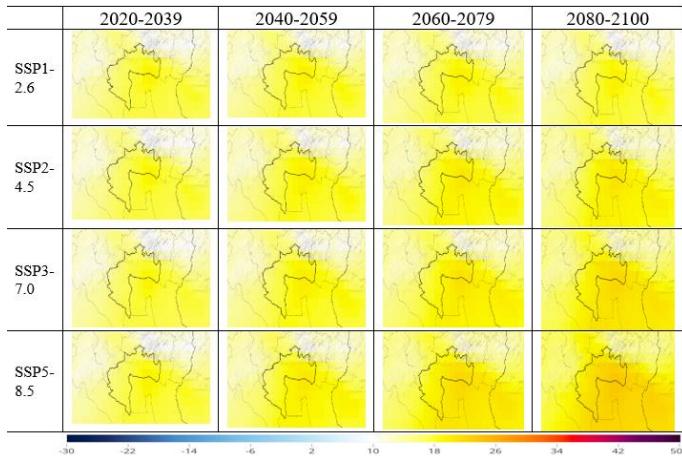
- Water resources: through changes in evapotranspiration and irrigation demand.
- Agricultural systems: through heat stress and altered crop growth conditions.
- Urban environments: through increased heat load and cooling demand.
- Environmental infrastructure: through performance limits under extreme temperatures.

## **2. SPATIO-TEMPORAL ANALYSIS OF TEMPERATURE FUTURE CLIMATE PROJECTIONS**

The analysis of average annual minimum surface air temperature highlights a persistent and spatially coherent warming trend across all SSP scenarios. In the near-future period (2020–2039), minimum temperature increases are relatively modest, particularly under SSP1-2.6, reflecting the delayed response of nighttime temperatures to emission reductions and the thermal inertia of the climate system (Asadollah et al., 2021; Hadi et al., 2024; Onyutha et al., 2021). As projections progress toward mid-century and late-century periods, minimum temperatures increase more rapidly, especially under SSP3-7.0 and SSP5-8.5.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

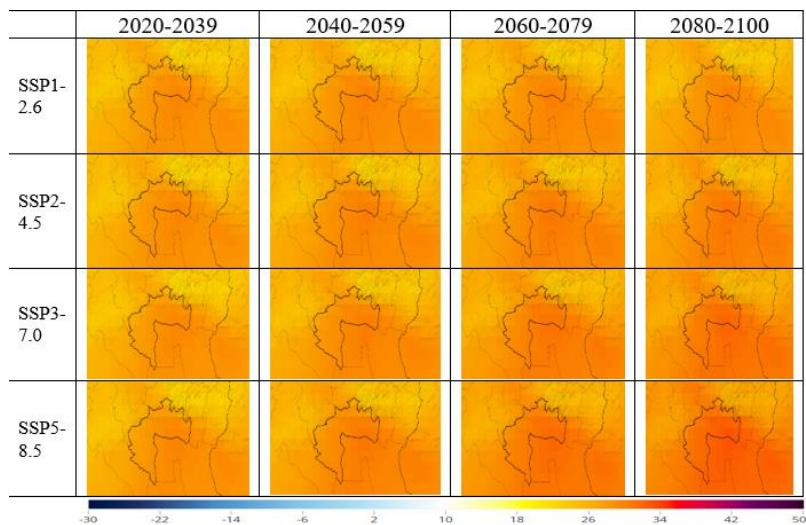
By 2080–2100, heat maps indicate a widespread elevation of nighttime temperatures across the entire Biskra region, suggesting a structural modification of nocturnal thermal regimes rather than isolated anomalies (Haq et al., 2023; Kamruzzaman et al., 2024; Ullah et al., 2022).



**Figure 1.** Evolution of Average Minimum Surface Air Temperature (Annual)

This trend has profound implications. Elevated minimum temperatures reduce nighttime cooling, which is essential for dissipating daytime heat accumulated in soils, vegetation, and built structures (Shi et al., 2021; Zhu & Burney, 2022). This phenomenon leads to increased baseline evapotranspiration rates, higher nocturnal water losses in irrigated systems, and reduced efficiency of passive cooling strategies in urban environments. In oasis-based agricultural systems, warmer nights may disrupt crop respiration cycles and reduce overall productivity, thereby increasing reliance on engineered irrigation and climate control solutions (Gabr et al., 2024; Jiao et al., 2024; Mueller et al., 2015).

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION



**Figure 2.** Evolution of Average Mean Surface Air Temperature (Annual)

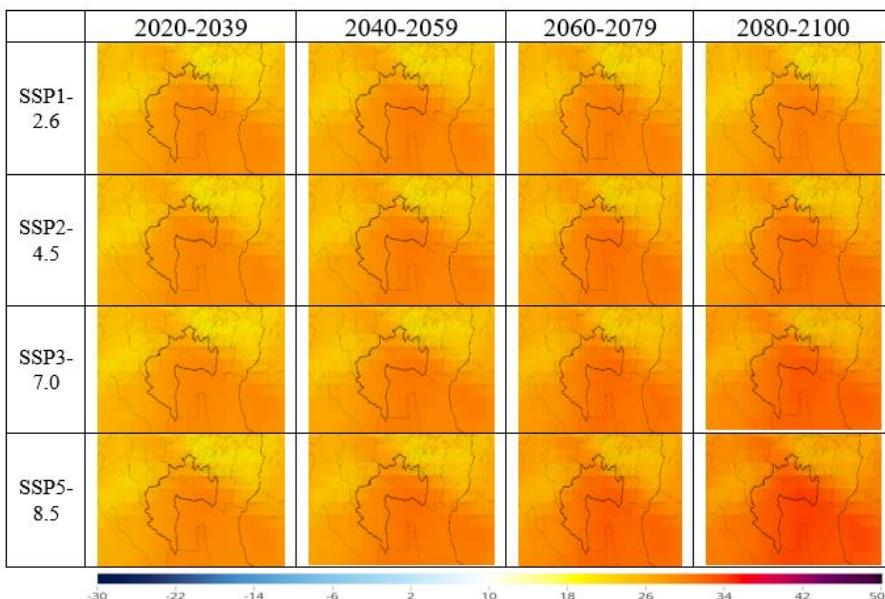
Projected changes in average annual mean surface air temperature reveal a clear upward shift in baseline climatic conditions across all scenarios. While differences among SSPs are relatively limited in the early projection periods, a strong divergence emerges after 2040, highlighting the cumulative effect of greenhouse gas emissions on regional climate (Almazroui et al., 2021; Odunmorayo et al., 2025).

Under SSP1-2.6, mean temperature increases tend to stabilize toward the end of the century, indicating the potential effectiveness of mitigation strategies in limiting long-term warming (Li et al., 2022). In contrast, SSP2-4.5 and SSP3-7.0 exhibit sustained increases, while SSP5-8.5 shows a pronounced and continuous warming trajectory through 2100 (Gatien et al., 2024; Tonelli et al., 2021).

Rising mean temperatures represent a chronic stressor that affects system performance over extended periods rather than through episodic events. Elevated baseline temperatures alter soil moisture dynamics, accelerate organic matter decomposition, and intensify salinization processes in irrigated lands (Haj-Amor et al., 2017; Hamidov et al., 2020; Nilahyane et al., 2023). These changes reduce water-use efficiency and challenge the sustainability of existing agricultural and water management infrastructures (Alkaff et al., 2025; Ferreira et al., 2024).

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

Furthermore, mean temperature increases necessitate the reassessment of engineering design criteria, including assumptions related to average water demand, crop water requirements, and thermal performance of materials (Chouhan et al., 2023; Nikolaou et al., 2020). Integrating projected mean temperature shifts into environmental impact assessments becomes essential for avoiding maladaptation and ensuring long-term system resilience (Aryal et al., 2019; Xu et al., 2025).



**Figure 3.** Average Maximum Surface Air Temperature (Annual)

The most critical results emerge from the analysis of average annual maximum surface air temperature. Heat maps consistently indicate a strong intensification of daytime extreme temperatures under intermediate and high-emission scenarios, with particularly severe conditions projected under SSP5-8.5(Chapman et al., 2024; H. Chen et al., 2022). From mid-century onward, maximum temperatures increase sharply, suggesting a future climate characterized by more frequent, prolonged, and intense heatwaves (Ha et al., 2022; Mishra et al., 2017).

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

In the Biskra region, where current summer temperatures already approach physiological and technical thresholds, additional warming significantly amplifies environmental and engineering risks (Bernacchi et al., 2025; Liu et al., 2025).

Extreme daytime temperatures increase irrigation losses through evaporation, reduce crop photosynthetic efficiency, and heighten livestock mortality risks. In urban environments, higher maximum temperatures exacerbate heat island effects, increase cooling energy demand, and strain water–energy systems (K. Chen et al., 2023; Huang et al., 2019; Parkes et al., 2022). Environmental infrastructure, including water distribution networks and treatment facilities, may experience reduced operational efficiency or accelerated material degradation under extreme heat conditions (Lin & Li, 2025; Sperling & Berke, 2017). These findings underscore the necessity of designing environmental systems capable of withstanding extreme thermal loads, rather than relying solely on historical climate norms (Kingsolver & Buckley, 2017; Tan et al., 2018).

### **3. COMPARATIVE ASSESSMENT OF SSP SCENARIOS**

A comparative analysis of the four SSP scenarios highlights the decisive role of socio-economic development pathways and climate policies in shaping future thermal conditions. SSP1-2.6 represents a mitigation-oriented pathway, where temperature increases remain relatively constrained, offering more favorable conditions for adaptation.

In contrast, SSP2-4.5 and SSP3-7.0 depict intermediate trajectories characterized by significant warming that may challenge existing adaptation capacities. SSP5-8.5 emerges as the most critical scenario, with extreme increases in minimum, mean, and maximum temperatures, potentially pushing the Biskra region toward unprecedented climatic conditions. The divergence between scenarios becomes particularly evident after 2050, emphasizing that future temperature outcomes are not inevitable but strongly dependent on global and regional policy choices.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

### **4. IMPLICATIONS FOR WATER RESOURCE MANAGEMENT IMPLICATIONS**

Understanding hydrological processes in response to climate change is vital for establishing sustainable environmental management strategies, as changes in temperature, evaporation, and water demand significantly affect agriculture, energy production, and water usage in watersheds (Chanie, 2024; Hyandyne et al., 2018). Water resource managers must now account for fundamental shifts in water availability patterns, where approximately 40% of basin areas that currently provide suitable conditions for surface irrigation systems may see reduced potential irrigable areas due to future climate change (Gondim et al., 2018; Masia et al., 2018).

The complexity of water management under climate change extends beyond simple supply and demand calculations. Global warming impacts on hydrological systems vary significantly from place to place, with some arid areas becoming wetter while previously semi-humid areas become drier (Elnashar & Elyamany, 2022; Teklay et al., 2021). This spatial variability requires region-specific management approaches, particularly as increased atmospheric CO<sub>2</sub> changes vegetation water use efficiency and breaks existing correlations between hydrology and ecology (Biazar et al., 2025; Islam et al., 2025).

Effective water resource management now requires integrated analytical frameworks that address the water-energy-food nexus, allowing managers to formulate coordinated strategies across multiple sectors (Borge-Diez et al., 2022). These frameworks must consider scenarios that constrain water use at the basin level to curb demand for multiple uses, which translates into significant impacts on agricultural water allocation across different crops (Meng et al., 2025).

The management challenge is particularly acute in arid regions where water resources are already becoming scarce due to precipitation decreases and temperature increases (Bastola et al., 2023). Reduced precipitation will decrease river flows while increased evaporation from drier atmospheres reduces water available in reservoirs for irrigation (Hoek Van Dijke et al., 2022).

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

To address these challenges, integrated watershed management systems and rehabilitation of forests and water bodies must be implemented to mitigate climate change effects and water shortage (Guirado et al., 2022; Hund et al., 2021). Interestingly, irrigation practices themselves can modify local climate conditions and water management outcomes. In hyper-arid regions with extensive irrigation, the cooling effect from irrigation can decrease evapotranspiration trends by reducing local temperatures and increasing relative humidity (Qiu et al., 2024). Understanding how evapotranspiration responds to both climate change and agricultural irrigation provides guidance for determining effective water resource measures for adapting to global change (Chen & Dirmeyer, 2019; Pool et al., 2021).

## **5. EXTREME TEMPERATURE EVENTS AND HEAT WAVE PROJECTIONS**

The IPCC framework establishes that extreme temperature events will intensify systematically with each degree of global warming. The frequency of hot temperature extremes, droughts, heavy precipitation events and tropical cyclones all increase with each additional 1°C of global warming, while rising temperatures will result in more frequent and more intense wet and dry weather events (Tye et al., 2022). This relationship creates a direct link between emission scenarios and the severity of extreme heat events that will affect infrastructure systems. Multi-model ensemble projections reveal a fundamental transformation in heat wave characteristics throughout the 21st century, with increasingly frequent and prolonged events that manifest in more localized patterns. Under high-emission scenarios, some regions may experience a threefold increase in event duration, with intensity potentially rising by 1.56°C by the late 21st century (Ortiz et al., 2019). Even under the most moderate SSP1-2.6 scenario, substantial changes are projected by the late 21st century, with heat wave frequency increasing by 146% and accumulated area expanding by 37%, while heat wave intensity reaches approximately 0.88°C above the threshold (Goyal et al., 2023). The progression from moderate to extreme emission scenarios shows dramatic escalation in heat wave characteristics.

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

Under SSP3-7.0, heat wave frequency is projected to increase by 37% by the late 21st century, with intensity potentially reaching 1.49°C above the threshold. Under the most extreme SSP5-8.5 scenario, heat wave frequency reaches 48 events per year, while projected decreases in heat wave area and moving speed indicate a fundamental shift toward more persistent and localized extreme heat events (Oliver et al., 2019). This transition toward stationary, intense heat events poses particular challenges for infrastructure systems that must maintain performance during prolonged exposure to extreme temperatures.

### **6. AGRICULTURAL IMPLICATIONS IN ARID ZONES**

The projected temperature increases under SSP scenarios will have severe consequences for agricultural systems in arid zones, fundamentally altering water balance and crop requirements. Evapotranspiration patterns show concerning trends, with arid regions including the Sahara Desert experiencing declining ET trends by the end of the 21st century, with larger decreases under SSP5-8.5 compared to SSP1-2.6 scenarios. The seasonality of evapotranspiration is also expected to intensify, with SSP5-8.5 and SSP3-7.0 projecting higher ET seasonality than the lower emission scenarios (Nooni et al., 2021).

Water stress and aridity are projected to increase dramatically across all scenarios. Research shows that aridity will increase under all scenarios by mid-century, with longer-term projections indicating different stabilization points: moderate scenarios (RCP4.5, SSP2-4.5, SSP3-7.0) stabilizing around SPEI values of -1.5, while extreme scenarios (RCP8.5, SSP5-8.5) project values of -2 or less by 2100 (Rakhmatova et al., 2024). Global analysis reveals that the Aridity Index has already declined from 0.49 (1900-1980) to 0.47 (1990-2015) and is projected to further decrease to 0.44 by 2040, indicating continued aridification (Malpede & Percoco, 2025). The expansion of arid conditions poses significant challenges for agricultural production. The area classified as arid or hyper-arid is projected to increase from 18,000 cells (1900-1980) to nearly 21,000 by 2040, representing a 16-percentage point increase in the world's land surface (Malpede et al., 2025).

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

This expansion will have adverse effects on agricultural production and economic development in affected regions (Malpede & Percoco, 2025).

Irrigation water requirements will increase substantially under climate change scenarios. Studies from arid regions show that annual average evapotranspiration is expected to increase by 0.35 mm/day (6%) under SSP2-4.5 and 0.7 mm/day (12%) under SSP5-8.5 by the 2100s. The growth in irrigation water requirement is projected to rise progressively under SSP5-8.5, increasing by 2.7% in the 2040s, 6.5% in the 2060s, 8.5% in the 2080s, and 12.4% in the 2100s compared to current conditions (Gabr, 2023).

These projected changes highlight the urgent need for adaptation strategies in arid agricultural systems. Without mitigation measures, warming could reach 4°C by the end of the century, worsening current conditions and necessitating comprehensive socio-economic and environmental impact studies to develop appropriate adaptation measures (Taibi et al., 2020). The findings provide crucial information for water resources managers to develop specific measures to mitigate extreme events in regions most affected by climate change (Nooni et al., 2021).

## **7. REGIONAL CONSIDERATIONS FOR BISKRA ALGERIA**

Biskra, located in Algeria's arid Saharan region, will experience particularly severe thermal stress under projected climate scenarios. Local studies using physiological temperature (PT) indices show alarming trends, with gradual increases in PT values starting from 2020 and progressively escalating through 2080 during summer seasons. The projections indicate extreme thermal heat-stress levels, with PT index averages showing increases of +5.9°C between 2020 and 2050, and +7.7°C by 2080, meaning no comfortable thermal stress zone is expected during 2080 (Matallah et al., 2021). Regional climate modeling across multiple time periods confirms consistent warming patterns that will affect Biskra and similar arid regions. Analysis of near future (2021-2040), middle future (2041-2060), and far future (2061-2080) periods under SSP2-4.5 and SSP5-8.5 scenarios demonstrates that both minimum and maximum temperatures will increase across all sub-basins in arid regions, while precipitation decreases in most areas (Sadeghi et al., 2022).

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

The temperature projections show universal increases across all latitudes and SSP scenarios, with SSP5-8.5 consistently showing the highest temperature increases compared to other scenarios. Different Global Climate Models (GCMs) show varying projections, with MIROC6 generally producing higher temperature projections for maximum, minimum, and average temperatures, while model variability differs across temperature metrics (Song et al., 2023). These regional considerations highlight that Biskra's location in Algeria's arid zone places it among the most vulnerable areas to climate change impacts, requiring urgent adaptation planning for agricultural systems and human thermal comfort.

## **CONCLUSION**

This chapter has analyzed projected changes in surface air temperature in the Biskra region throughout the twenty-first century, utilizing SSP-based climate scenarios and spatial heat map analysis. By integrating projections of minimum, mean, and maximum temperatures with an environmental engineering perspective, the chapter offers a comprehensive assessment of both chronic and extreme thermal stresses anticipated in arid environments under climate change. The findings clearly indicate a persistent and significant warming trend across all scenarios and time horizons. Minimum temperatures are projected to rise steadily, diminishing nocturnal cooling and increasing baseline thermal stress. Mean temperatures are expected to shift upward, redefining the climatic baseline that governs long-term environmental processes and system performance. Maximum temperatures exhibit the most critical changes, with a strong intensification of daytime extremes and heatwave risks, particularly under intermediate and high-emission scenarios.

A key finding of this chapter is the pronounced divergence between SSP scenarios after mid-century. Mitigation-oriented pathways such as SSP1-2.6 offer relatively manageable thermal conditions, whereas high-emission pathways, especially SSP5-8.5, project temperature levels that may exceed the adaptive capacity of existing environmental systems and infrastructure. This divergence underscores the decisive role of socio-economic choices and climate policies in shaping future environmental constraints at the regional scale.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

From an environmental engineering standpoint, the projected temperature changes imply profound implications for water resource management, agricultural sustainability, urban thermal comfort, and infrastructure resilience. Rising temperatures intensify evapotranspiration, increase water demand, reduce system efficiency, and exacerbate heat-related risks for ecosystems and human populations. These impacts underscore that climate change is not a distant or abstract concern but a central design constraint for present and future environmental engineering projects. In conclusion, this chapter demonstrates that integrating climate projections into environmental engineering analysis is essential for ensuring long-term sustainability and resilience in arid regions. Heat maps and SSP-based scenarios provide valuable decision-support tools for anticipating future thermal conditions and informing adaptive strategies. The case of Biskra illustrates how climate-informed environmental engineering can contribute to proactive planning, risk reduction, and sustainable development under a warming climate.

### ***Recommendations for Environmental Engineers***

Based on the projected temperature changes and their implications discussed in this chapter, several recommendations can be formulated to support climate-resilient environmental engineering in arid regions such as Biskra.

- **Integrate Climate Projections into Engineering Design:** Environmental engineers should systematically incorporate SSP-based temperature projections into the design and evaluation of water, agricultural, and urban infrastructure. Design standards based solely on historical climate conditions are no longer sufficient and may lead to maladaptation under future thermal regimes.
- **Adopt Risk-Based and Scenario-Oriented Planning:** Engineering projects should be evaluated under multiple climate scenarios, including high-emission pathways, to identify worst-case conditions and ensure system robustness. Scenario-based planning enables the development of flexible and adaptive solutions capable of performing under a wide range of future climates.

## *CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION*

- Enhance Water-Use Efficiency and Thermal Resilience: Given the projected increase in evapotranspiration and water demand, priority should be given to improving irrigation efficiency, reducing water losses, and adopting advanced water-saving technologies. Engineering solutions should aim to optimize water–energy interactions and minimize thermal vulnerability.
- Promote Climate-Resilient Agricultural and Oasis Systems: Environmental engineers working in agricultural contexts should support the adoption of heat-tolerant crop varieties, optimized irrigation scheduling, and soil management practices that reduce thermal and water stress. Protecting and enhancing oasis ecosystems should be considered a strategic adaptation measure in arid regions.
- Mitigate Urban Heat through Environmental Design: Urban environmental engineering should emphasize heat mitigation strategies, including green infrastructure, shading, reflective materials, and optimized urban layouts. These measures can reduce heat island effects and improve thermal comfort while lowering energy demand for cooling.
- Strengthen the Water–Energy–Climate Nexus Approach: Future environmental engineering projects should adopt an integrated water–energy–climate nexus perspective to avoid trade-offs and maximize co-benefits. Cooling demand, water supply, and energy consumption should be addressed as interconnected challenges rather than isolated sectors.

**CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL  
NUTRITION**

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*CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL  
NUTRITION*

**CHAPTER 2**  
**WHEY AS AN ADDITION IN THE DIET AND ITS**  
**EFFECT ON THE LIVER**

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## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

### INTRODUCTION

Enzymes are catalysts of biological origin. It is a special class of proteins that catalyze chemical reactions in biological systems. In the body of man, animals, plants, a whole series of metabolic processes of decomposition and synthesis take place (Džekova, 2006).

Parameters used to examine liver function are enzymes: ALT-alanine aminotransferase, AST-aspartate aminotransferase, SDH-sorbitol dehydrogenase, LDH-lactate dehydrogenase, GLDH-glutamate dehydrogenase,  $\gamma$ GT-gamma glutamyl transferase, ALP-alkaline phosphatase,  $\gamma$ - globulins, iron, copper, ceruloplasmin and others.

Alanine aminotransferase, found in the serum and in some tissues, is mostly associated with the liver. This enzyme catalyzes the transfer of an amino group from alanine to  $\alpha$ -ketoglutarate, yielding pyruvate and glutamate. The level of this enzyme is commonly measured to diagnose liver function, along with aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, and bilirubin. Aspartate aminotransferase-AST is similar to ALT which is also associated with liver cells and is elevated when there is acute liver damage. This enzyme is present in erythrocytes and heart muscle, skeletal muscle, kidney and brain tissue.

$\gamma$ GT ( $\gamma$ -glutamyltransferase) is an enzyme involved in the transfer of the  $\gamma$ -glutamyl residue from  $\gamma$ -glutamyl peptides to amino acids, H<sub>2</sub>O<sub>2</sub>, and other small peptides. This enzyme is found primarily in the tissues of the kidneys, brain, prostate, pancreas and liver.

The liver is one of the largest organs in the human body and possesses the power of self-regeneration. Over 500 (anabolic and catabolic) processes take place in it, and that is why they call it the body's laboratory. It takes place: 1) metabolism of proteins, carbohydrates, fats, 2) bile is created, 3) numerous substances are deposited (vitamins, iron, amino acids, glycogen), 4) it has a detoxification function - used hormones, ingested xenobiotics, drugs it also conjugates various chemicals, making them water-soluble, and 5) finally, it excretes them, that is, it has an excretory function. Liver diseases can be divided into: viral liver diseases; autoimmune liver diseases; toxic liver diseases; hereditary liver diseases; oncological diseases of the liver (Mathias Karlsson, Saulieus Satas, Helen Porter, Marianne Thoresen, 2009).

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

A large number of biochemical processes take place in the liver, therefore the parameters and tests used to examine its function are numerous and diverse. The tests can serve as a screening for previous viral diseases, help monitor the progress of a disease, monitor the effect of the received therapy and determine the size of the damage. In recent years, the ingredients of milk represent a functional food, because their use has a great effect on health (Marshall, 2004). Whey in its original form is a liquid that contains less than 1% protein and over 93% water. After several stages of processing (a more sophisticated process) it can be refined and completely converted into proteins. In milk there are two completely different types of proteins, casein and whey proteins (in the ratio 80:20%). Whey is a byproduct of cheese production, which was previously considered waste. It contains  $\beta$ -lactoglobulin,  $\alpha$ -lactoalbumin, serum albumin, lactoferrin, immunoglobulins, lactoperoxidase, glycomacropeptides, lactose and minerals. Whey proteins strengthen the immune system by helping the body produce the antioxidant glutathione (Marshall, 2004).

Glutathione protects against free radicals, pollution, toxins and infections. Adding whey protein to the diet can improve the health of people of all ages. Whey has been considered a medicinal drink since ancient and medieval times. Due to the wide spectrum of essential and non-essential amino acids, minerals, fats and biologically active proteins, whey is widely used in the treatment of various diseases. Whey is still an enigma and underutilized even in the 21st century and is interesting for researchers, for production and marketing. The emphasis is on the fact that it is a medicinal liquid that contains 16 types of proteins, 8 minerals, 7 vitamins, up to 23 amino acids, up to 11 enzymes and many others. substances for the development of the animal world. Whey is rich in minerals: calcium, which is in a good ratio with phosphorus, as well as a good ratio of potassium with sodium, contains Cl, Cu, Zn, Fe, Mn and Mo in traces that maintain cell tone, prevent high blood pressure, stroke, heart attack. The healing power of whey is still being researched, but it is generally known that the main problem in the body occurs when the power to regenerate organs or life processes disappears, which in regular conditions takes years, with intensive nutrition with whey in all its original or derivative forms leads to regeneration (Healthy nutrition, 2009).

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

The use of whey in the diet is also related to the fattening of pigs and the improvement of meat quality when adding lysine-amino acid present in fodder, which according to the expert literature are very different. (reference).

### **1. CHEMICAL COMPOSITION OF BLOOD PLASMA AND SERUM IN PIGS**

Blood plasma is a blood liquid without the presence of blood cells that have been removed by centrifugation of citrated blood. Blood serum is a clear yellowish liquid, which is obtained after coagulation of blood through centrifugation. It can also be obtained by centrifugation of defibrinated blood. From the total blood, plasma accounts for 55-70%. The chemical composition of the blood plasma in individual species of animals is very similar, but there are significant quantitative differences in the inorganic and organic ingredients.

A large number of organic compounds are found in the blood plasma. There are two types: nitrogen compounds, which are the most, and non-nitrogen compounds, which are found in small quantities, but have a significant physiological function.

In the blood plasma and serum, proteins are the most represented of the nitrogenous compounds. Of the total amount of organic substances in plasma, which is about 85 g/L plasma, 60-80 g are proteins. There are about 200 different proteins that can be divided into simple and complex. Simple ones include fibrinogen, serum albumins and serum globulins, and complex  $\alpha$  and  $\beta$  lipoproteins and glycoproteins. The table shows the main proteins of blood plasma and serum in pigs (Petkov, 2000).

**Table 1.** Proteins in plasma and serum (g/L) in some domestic animals

Animal	Total proteins (plasma)	Fibrinogen	Total proteins (serum)	Albumins	Globulins
<b>Pig</b>	68,0	5,0	63,0	20,3	32,7
<b>Horse</b>	68,4	3,4	65,0	32,5	32,5
<b>Cattle</b>	83,2	7,2	76,0	36,3	39,7
<b>Sheep</b>	57,4	3,6	53,8	30,7	23,1
<b>Goat</b>	72,7	6,0	66,7	39,6	27,1
<b>Dog</b>	67,2	5,2	62,0	35,7	26,3

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

Proteins in blood plasma are the most important part of organic substances, so their role is multiple and very significant. Their physiological role consists in: colloid-osmotic regulation, buffer role, blood coagulation, defense role, transport role and metabolism.

Inorganic ingredients consist of water and inorganic salts. Water is about 90-92% of the blood plasma, and part of it is free, in which the salts of low molecular weight compounds are dissolved. The other part is bound to the proteins, namely 50 g of water per 100 g of proteins, most of the water is bound to the serum albumins. About 0.9% of blood plasma and serum are inorganic salts. The salts present in the blood are divided into cations and anions, and the more important ones in pigs are shown in the table (Petkov, 2000).

**Table 2.** Important inorganic constituents in the serum of some domestic animals

Ingredients	Measurement units	pig	Cattle	Sheep	Horse	Dog
<b>Sodium</b>	mmol/l	146,0	140,0	144,0	140,0	144,0
<b>Potassium</b>	mmol/l	5,2	4,4	4,7	4,7	5,2
<b>Calcium</b>	mmol/l	2,5	2,5	2,5	2,5	2,7
<b>Magnesium</b>	mmol/l	1,2	1,2	1,0	1,1	1,0
<b>Chlorides</b>	mmol/l	104,0	104,0	104,0	101,5	110,0
<b>Iron</b>	μmol/l	32,0	18,0	22,0	22,0	25,0
<b>Copper</b>	μmol/l	34,0	13,0	12,6	20,0	25,0
<b>Zinc</b>	μmol/l	11,5	23,0	-	15,6	50,0
<b>Total iodine</b>	μmol/l	120-430	200-500	230-630	230-470	160-350

Sodium is an important regulator of the constancy of the osmotic pressure of blood plasma and extracellular fluids and as an activator of several enzymes and for the synthesis of hydrochloric acid in the stomach. The physiological function of potassium consists in regulating the permeability of the cell membrane, as well as the excitability of nerves, muscles and muscle contraction.

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

Calcium reduces the permeability of the cell membrane, irritation of nerves, muscles and muscle contraction, is an activator of enzymes, participates in blood coagulation and other functions.

Magnesium reduces the excitability of nerves and muscles and is an activator of several enzymes.

Chlorides are quantitatively the most important anions of the blood plasma and have the same physiological function as NaCl. Bicarbonates take part in the acid-base balance

In addition to inorganic phosphates, the majority of phosphorus in the blood plasma is found in the form of organic phosphates. And sulfur is also found in a larger part in the composition of proteins. Part of the sulfur is inorganic sulfur, and the other part is bound to the decay products in the intestines: phenol, indole, skatole and others.

## **2. CLINICAL ENZYMOLOGY**

Enzymes are biological molecules that catalyze chemical reactions. In enzymatic reactions, molecules at the beginning of the reaction, called substrates, are converted into different molecules called products.

Clinical enzymology is a discipline that studies and tests enzyme activity in serum, plasma, urine and other body fluids in order to aid in the diagnosis and prognosis of disease and to obtain a picture of the abnormal function of an organ (J. Jerry Kaneko, John W. Harvey, Michael L. Bruss, 2008). There are several factors that affect the activity of enzymes in serum.

- One of those factors is the size of the organ and the concentration of enzymes in the tissue. The role of these factors on the magnitude of enzyme activity in the blood is very clear. Organs with a high concentration of enzymes have the potential to cause a high increase in serum enzyme activity during disease. For example, the intracellular versus extracellular concentration gradient of hepatocellular alanine aminotransferase (ALT) is 100,000:1. Hepatocyte injury causes a significant increase in serum ALT activity. The greater the concentration gradient of enzymes or proteins between cells and the interstitial space, the faster the translocation of a significant amount of enzymes (Mair, 1999).

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

- The location of the cells, it depends on whether the enzymes will be released into the blood, urine, bile or other body fluids. That's why alkaline phosphatase (ALP), which is located on the luminal surface of enterocytes, when these cells are injured, ALP is more in the stomach lumen than in the blood.
- Mechanism of release of cytoplasmic enzymes from the cell into the blood. A healthy membrane should be impermeable to macromolecules such as enzymes. But the increase of serum enzymes does not necessarily mean that it is a matter of necrosis, because the cytoplasmic enzymes can "leak" from the diseased cells through pores or as bubbles.
- Mechanism of release of enzymes bound to the membrane. These enzymes that are attached to membrane surfaces such as ALP and  $\gamma$ GT are released into the blood by an apparently different mechanism than the cytoplasmic enzymes.
- Enzyme induction. The change in enzyme activity in the serum may in some cases be caused by changes in the production of enzymes by the cells rather than by injury to the cells. Such induction can be the result of hormonal changes, pathophysiological events such as cholestasis, or be caused by drugs.

### ***Alanine Aminotransferase***

Alanine aminotransferase (ALT) is an enzyme that belongs to the class of transferases. It catalyzes the transfer of the amino group from alanine to  $\alpha$ -ketoglutarate to form glutamate and pyruvate. The old terminology was serum glutamate-pyruvate transaminase (SGPT or GPT). ALT, like other transaminases, participates in amino acid catabolism and nitrogen transport in organs.

ALT activity has been found in several organs, but the magnitude of the activity varies between different species. In dogs, the activity is highest in the liver, about four times, in contrast to the heart and skeletal muscles. It is similar in cats, but in pigs, cattle and horses the difference is not so great. Thus, according to the tissue concentration of ALT, elevated serum ALT activity is specific for liver disease in dogs and cats, but does not offer specific detection for horses and cattle.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

The half-life of ALT in the blood has not been precisely determined, although the circulation time is sufficiently long to evaluate liver disease and to release ALT into the blood several hours to days.

Serum ALT has been recognized as a marker for hepatocellular diseases since 1950. The ratio between serum ALT and ALP is much higher in cases of hepatic necrosis than in cholestasis, which means that a general conclusion can be interpreted with the ratio of these two enzymes. Elevated serum ALT levels also occur with other disorders such as hypoxia, metabolic diseases such as lipidosis, nutritional disorders such as copper toxicosis. The increased level also occurs when taking some drugs, which cause hepatocellular toxicity.

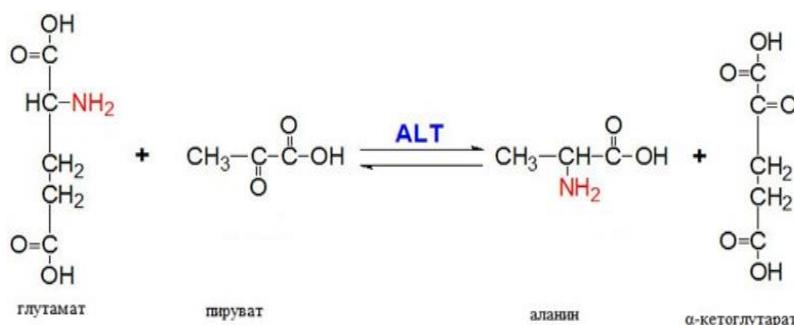


Figure 1. Reaction catalyzed by alanine aminotransferase

An increase in serum ALT has been observed in dogs and cats with diseases such as diabetes mellitus, hyperthyroidism, hyperadrenocorticism and hypothyroidism. For example, 163 (78%) dogs with diabetes mellitus have elevated serum ALT (Hess R.S., Saunders H. M., Van Winkle T.J., Ward C. R., 2000).

All research leads to the conclusion that high values of serum ALT are mostly specific for liver disease, but sometimes it can also be the result of myocyte disease. Therefore, the clinical application of ALT tests is mainly limited to the evaluation of liver disorders. There are higher increases in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders. In acute inflammatory conditions of the liver, elevations in ALT are often higher than those in AST and tend to remain elevated for a longer time as a result of the longer half-life of ALT in serum.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

### Aspartate Aminotransferase

Aspartate aminotransferase (AST) is an enzyme that belongs to the family of transferases. It is often referred to as transaminase and is involved in the transfer of the amino group from L-aspartate to 2-oxoglutarate to yield oxaloacetate and glutamate. The old terminology is glutamate-oxaloacetic transaminase (SGOT or GOT). Pyridoxal-5'-phosphate functions as a coenzyme.

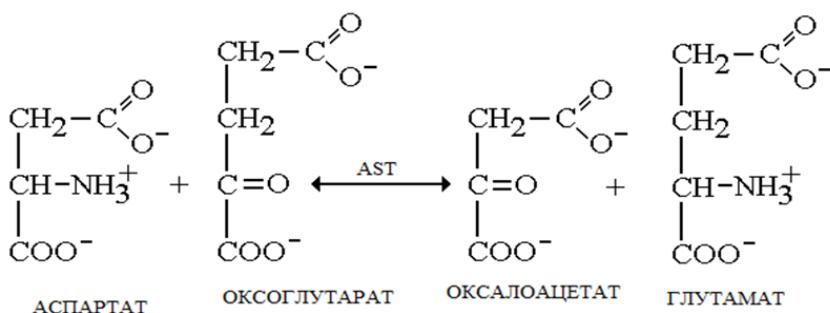


Figure 2. Reaction catalyzed by aspartate aminotransferase

AST is widely distributed in human tissues. The highest and almost the same concentration is found in the liver, skeletal muscles and heart muscle, and in small amounts it is found in the kidneys, pancreas and erythrocytes. AST is located in the cytosol, but it is present in larger amounts in the mitochondria. The cytoplasmic isoenzyme is the predominant form in serum. In disorders that cause cell necrosis, such as liver cirrhosis, the mitochondrial form can be significantly increased. It is used for screening tests for both the liver and the heart. This enzyme has a longer blood half-life than sorbitol dehydrogenase and creatine kinase and is stable at room temperature. AST levels begin to rise within six to eight hours, peak within 24 hours, and generally return to normal within five days (Michael L. Bishop, Edward P. Fody, Larry E. Schoeff, 2005). Elevations of AST are often seen in pulmonary embolism. In heart failure it is also elevated, possibly reflecting liver involvement as a result of inadequate blood supply to that organ.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

Because serum AST cannot differentiate between hepatocellular or myocyte disease, further tests involving organ-specific enzymes such as SDH or CK are needed. High values of AST and SDH indicate acute or active hepatocellular disease.

### $\gamma$ -glutamyltransferase

$\gamma$ -glutamyltransferase ( $\gamma$ -GT) is an enzyme involved in the gamma glutamyl cycle where it catalyzes the transfer of  $\gamma$ -glutamyl groups from  $\gamma$ -glutamyl peptides such as the tripeptide glutathione to other peptides, amino acids and water. In most biological systems glutathione serves as a donor of  $\gamma$ -glutamyl.

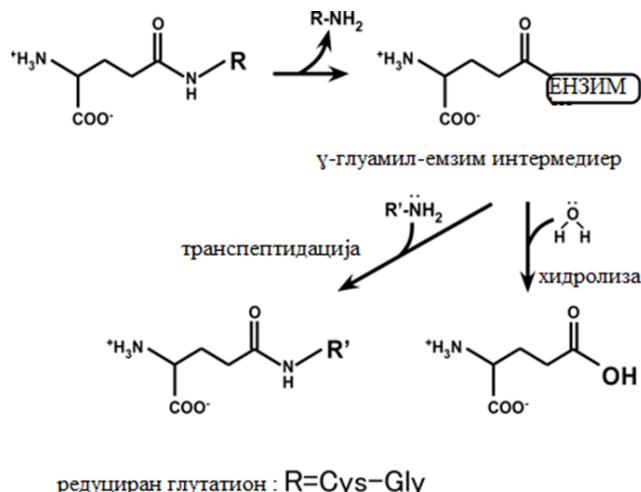


Figure 3. Reaction catalyzed by  $\gamma$ -glutamyltransferase

The tissue origin of  $\gamma$ -GT has been studied in several studies in numerous domestic animals and the highest concentration has been found in the kidney, pancreas, intestine and in the mammary glands of dogs, cattle and sheep, but less in the mammary glands of horses. A lower concentration of  $\gamma$ -GT was found in the liver, spleen, intestine and lung.  $\gamma$ -GT is a membrane-bound enzyme on the outer surface of cells and is linked to the cell membrane by hydrophobic transmembrane peptides.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

This enzyme is located on the surface of the proximal tubular cells of the kidneys, from where it spills into the urine in case of tubular disorders. The clinical application of the tests, however, is mainly aimed at evaluating disorders of the liver and biliary system. In the liver,  $\gamma$ -GT is primarily found in biliary epithelial cells and because of this location it is elevated in almost all hepatobiliary disorders. High values are observed during obstructions of the biliary system.

$\gamma$ -GT levels are also elevated in acute pancreatitis, diabetes mellitus, and myocardial infarction.  $\gamma$ -GT activity is useful in distinguishing the source of an elevated ALP level, as  $\gamma$ -GT levels are normal in skeletal disorders and during pregnancy. It is particularly useful in the evaluation of adolescent hepatobiliary disorders, as ALP activity will be elevated due to bone growth.

### 3. LIVER PHYSIOLOGY IN PIGS

The liver is located just behind the diaphragm. It receives both arterial and venous blood: the majority, about two-thirds through the portal vein, and one-third through the hepatic artery (a. hepatica). Almost one third of the minute volume of blood passes through the liver, which speaks of the importance of its role.

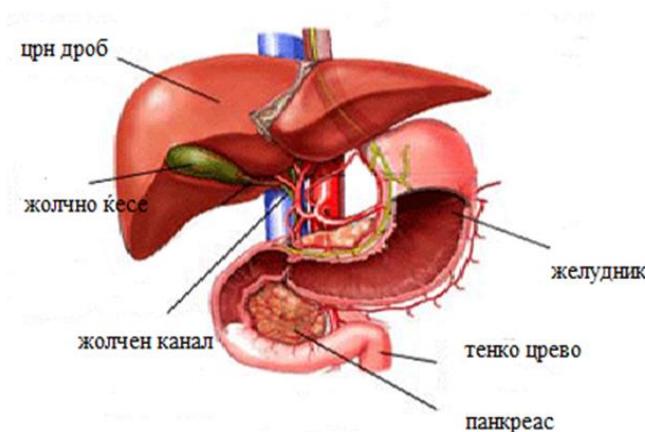


Figure 4. Position of liver and gallbladder

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

Venous blood comes from the stomach, intestines and pancreas through the portal vein. Hepatic capillaries are larger than normal capillaries and are called sinusoidal capillaries. The hepatic artery supplies blood to the bile ducts and connective tissue, provides the necessary partial pressure of oxygen in the blood passing through the liver parenchyma, and constitutes a reserve source of blood supply to the liver tissue (J. Jerry Kaneko, John W. Harvey, Michael L. Bruss, 2008).

In addition to being a blood depot in the body, the liver also circulates extracellular fluid. Much more lymph is synthesized in it than in other organs.

Liver cells (hepatocytes) constantly secrete bile, which enters the duodenum through the bile duct. Bile, due to the presence of bile acids in it, which participate in the digestion of fats, is considered a secretion of the liver. On the other hand, it also contains bile dyes and other substances that are end metabolic products unnecessary for the body and are thrown out, so it is also an excrement.

The liver produces cholesterol that is transferred to the blood and bile. In it, many hormones, toxins and drugs are transformed and inactivated (many substances are converted into water-soluble metabolites and thus are excreted through urine or bile). Most of the plasma proteins and coagulation factors and many other functions are produced in the liver.

The hepatocytes, which constantly secrete bile into the bile capillaries, which unite into larger bile ducts, from which the bile duct (ductus hepaticus) is finally formed. The gallbladder is a reservoir for bile. In ruminants and pigs, the bile does not concentrate much because digestion in their intestines is continuous, and this requires a constant flow of bile (Petkov, 2000).

### **4. LABORATORY ASSESSMENT OF LIVER FUNCTION**

The pathogenesis of liver diseases in domestic animals is complex, including acute and chronic forms of hepatitis, cirrhosis, obstruction of the bile duct, cholestasis, neoplasia and disorders of hepatic blood vessels. The frequency of these diseases varies between different species, depending on age, race and the environment, i.e. geographical location and diet. Laboratory tests are used to evaluate the severity of the liver disorder, establish a prognosis, and monitor clinical progress.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

Conventional tests for liver disease provide us with information about the integrity of hepatocytes (ALT, AST, SDH) and the status of the biliary system (ALP, GGT). Additional information is obtained from tests for albumin, total protein, triglycerides, cholesterol, glucose, and coagulation measurements (Zimmerman, 1999).

### **5. HEPATIC ENZYMES**

The presence of a liver disorder is often recognized by the elevated values of enzymes of hepatic origin in the serum. Serum enzymes whose values increase in hepatic necrosis are alanine aminotransferase (ALT), aspartate aminotransferase (AST), ornithine carbamoyltransferase (OCT), glutamyl dehydrogenase (GD), sorditol dehydrogenase (SDH), and arginase. Elevated serum values of alkaline phosphatase (ALP),  $\gamma$ -glutamyl transferase ( $\gamma$ -GT), and 5' nucleotidase (5'-ND) indicate cholestasis.

The main value of ALT and AST measurements is the detection of hepatocellular disorders and monitoring of clinical progress. Since the level of both enzymes increases in liver diseases, they have limited value in differential diagnosis.

#### ***Serum Alanine and Aspartate Aminotransferases as Indicators of Liver Disease***

The serum activity of these transferases is measured to detect any hepatocellular damage. These enzymes have a major role in gluconeogenesis and urea formation.

In the liver, ALT catalyzes the transfer of  $\alpha$ -amino nitrogen from alanine to  $\alpha$ -ketoglutarate forming pyruvate, which is used in gluconeogenesis (J. Jerry Kaneko, John W. Harvey, Michael L. Bruss, 2008). In muscle, ALT transaminates pyruvate to alanine, which transports unionized nitrogen from muscle to the liver for processing, the glucose-alanine cycle. ALT activity is higher in the liver than in other organs in dogs, humans, and rodents. AST activity is higher in the liver of all domestic animals and is routinely measured to evaluate liver disease. AST is also high in the kidney, heart, and skeletal muscle. 2.4.1.2.  $\gamma$ -glutamyl transferase as an indicator of liver disease.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

$\gamma$ -GT is a membrane-bound enzyme that catalyzes the transfer of  $\gamma$ -glutamyl groups from  $\gamma$ -glutamyl peptides such as glutathione to other amino acids or peptides. Glutathione and glutathione conjugates are the most widely distributed physiological substances.  $\gamma$ -GT is found mostly in the liver, kidney, pancreas and intestine. This enzyme is considered as a serum marker for diseases of the hepatobiliary system along with cholestasis (Hanigan, 1998).

### ***Serum Bilirubin and Bile Acids As Indicators of Liver Disease***

In animals, there is a lower level of total bilirubin in the serum. If the amount of total bilirubin is elevated, the serum acquires a distinct yellow color. In most methods of measuring bilirubin hemolysis interferes, it is more of a problem when hemolysis occurs due to helolytic anemia than when it is caused during blood collection. Blood samples must be protected from light, as the bilirubin level decreases.

Serum bilirubin may increase due to drug inhibition of uridine diphosphate glucuronosyltransferase or bilirubin transporter inhibitors (Zuker, S.D., Rouster, S.D., Green, P., 2001).

The synthesis of primary bile acids is the main pathway of cholesterol catabolism. In pigs, hyochol is produced in the liver and is the major bile acid (Schwarz, M., Russell, D.W., Dietschy, J.M., and Turley, S.D., 1998). Primary acids are conjugated with taurine or glycine and transported. In the duodenum and jejunum, they play an important role in the digestion and absorption of fats and other lipids, including liposoluble vitamins. In the thermal ileum, all types of bile acids, both primary and secondary, are absorbed and transported to the liver via the portal vein. Bile acids are recommended as an alternative test after bilirubin (Center, 1993).

### ***Serum Proteins As Indicators of Liver Disease***

A lot of proteins are synthesized in the liver, the analyzes of total proteins, albumin and globulin are usually performed. With a reduced diet, hypoproteinemia occurs, as well as with some hepatocellular inflammation. In some cases of liver damage, it also produces acute-phase proteins such as protein C, haptoglobin, and fibrinogen.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

By measuring protein C, it has been confirmed that it is a biomarker for assessing liver damage (Saha et al, 2007), and together with some other laboratory tests, its value in the recognition of some disorders and diseases of the liver has been shown (Toulza, 2006).

### **6. WHEY**

Whey is a clear yellowish liquid that separates when cheese is made. It represents 90% of the original weight of the milk used to make cheese. It contains between 6-6.4% solids, which is half of the total content in milk. In recent years, the constituents of milk have become recognized as functional foods, indicating that their use has direct and measurable effects on health. Whey is a by-product of the production of cheese and cream, which used to be considered waste. Milk contains two primary sources of protein, casein and whey protein.

Today, whey is a popular dietary food that provides antimicrobial activity, immunomodulation, improves muscle mass and prevents cardiovascular diseases and osteoporosis. Whey protein concentrates (80-95% protein), lactose-reduced whey, whey protein isolates, demineralized and hydrolyzed whey are commercially available. All these differ according to the amount of proteins, carbohydrates, immunoglobulins, lactose, minerals and fats in the final product. Table 3 describes several types of whey that are commercially available (Marshall, 2004).

The separation of whey depends on the production technology of the basic product, as well as on the quality of the milk used. In addition, processing methods such as filtration, centrifugation, and enzymatic treatment play a crucial role in determining the functional properties of whey. Variations in processing conditions can influence the bioavailability of nutrients and bioactive compounds present in whey. Therefore, both raw material quality and processing techniques are key factors affecting the nutritional and physiological value of whey products.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

**Table 3.** Types of commercially available whey proteins

Product description	Protein concentration	Mineral, fat and lactose content
Whey protein isolate	90-95 %	A little bit of everything
Whey protein concentrate	25-89 % (mostly 80%)	As protein content increases, fat, lactose and minerals decrease.
Hydrolyzed Whey	Variable Hydrolysis breaks the peptide bonds Larger proteins become small peptide fractions	Varies depending on the amount of protein
Undenatured Whey Concentrate Variable	Mostly between 25-89%	Low content of fat, lactose and minerals Where the natural protein structure is retained, there are more immunoglobulins and lactoferrin

### ***Whey Composition***

Mild whey contains the most lactose, protein and minerals. It is rich in riboflavin and pantothenic acid. Table 4 shows the average composition of whey (Fox, P. F., McSweeney, P. L. H. , 1998).

**Table 4.** Average composition of whey

Water	Около 93.8%
Lactose	5.0
Proteins	0.8
Minerals	0.55
Potassium	0.147
Calcium	0.043
Phosphorus	0.040
Sodium	0.060
Fats	0.30
Riboflavin	1.20 mg/kg
Niacin	0.85
Pantothenic acid	3.4

*CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL  
NUTRITION*

Thiamine	0.40
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## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

According to acidity, whey can be divided into three groups:

- Sweet whey, where the titration acidity ranges from 0.10 to 0.20%, and the pH value ranges from 5.8 to 6.6.
- Medium acid whey, where the titration acidity ranges from 0.20 to 0.40%, and the pH value from 5.0 to 5.8.
- Acid whey, where the titration acidity is greater than 0.40 and the pH value is less than 5.0. Titration acidity is expressed as a percentage of lactic acid.

About 50% of the dry matter of the milk passes into the whey, of which about 70% is lactose, depending on the acidity of the whey, the proteins are in the second place in order of representation, then the mineral substances, and finally the fats. In addition to protein, lactose, vitamins and minerals, whey also contains enzymes, hormones and growth factors (Hui, 1993).

### ***Mineral Substances***

Copper has been identified as an essential component of many metalloenzymes, including vital enzymes. Copper-containing enzymes are involved in the release of energy during respiration and the synthesis of structural proteins such as collagen. Zinc has multiple functions, such as stimulating insulin to absorb glucose from the blood. Iron is part of several metalloenzymes such as hemoglobin, lactoperoxidase, catalase, and participates in oxygen transfer. Lactoferrin can prevent some unwanted bacteria from binding to iron, thus inhibiting their growth in the gut. Iodine is part of thyroid hormones and plays an important role in the regulation of growth and development of newborns. Cobalt is found in vitamin B12, which prevents pernicious anemia (Fox, 2000).

### **6.1 Whey Proteins**

Whey is composed of several proteins including beta-lactoglobulin, alpha-lactalbumin, serum albumin (BSA), and glucomacropeptide (GMP). Whey protein contains all the essential amino acids and in higher concentrations compared to some vegetable protein sources such as soy, corn and wheat (Walzem RL, Dillard CJ, German JB, 2002).

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

The representation of free amino acids in sweet whey is four times higher than in the initial milk, and in sour whey by about ten times. Compared to other protein sources, whey has a high concentration of branched-chain amino acids (BCAAs)—leucine, isoleucine, and valine. VCAA, especially leucine, are very important factors for tissue growth and repair. Leucine has been identified as a key amino acid in protein metabolism (Anthony Jc, Anthony TG, Kimball SR, Jefferson LS, 2001). The sulfur-containing amino acids cysteine and methionine in whey are also present in high concentrations, and they contribute to the improvement of immunity through intracellular conversion to glutathione (Marshall, 2004).

Proteins in milk have many roles that make dairy products and other products containing these components so valuable.

These proteins are not sensitive to the action of acids or enzymes, so they remain unchanged during coagulation and after removing the casein curd, they pass into the whey. That is why the amount of protein in sweet and sour whey is similar (Presilski, 2004). Table 5 shows some whey proteins, their representation and their advantages.

**Table 5.** Components found in whey proteins

Whey components	% of whey proteins	Advantages
β-Lactoglobulin	50-55%	Source of essential and branched chain amino acids
α-Lactalbumin	20-25%	The primary protein found in human milk. Source of essential and branched chain amino acids
Immunoglobulins	10-15%	The primary protein found in colostrum. Immunomodulation
Lactoferrin	1-2%	Antioxidant, antibacterial, antiviral and antifungal action. It allows good bacteria to grow. It is found in body fluids
Lactoperoxidase	0.50%	Inhibits the growth of bacteria
Serum albumin	5-10%	Source of essential and branched chain amino acids
Glucomacropeptide	10-15%	Source of branched chain amino acids. It binds with aromatic amino acids

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

### **6.2 Mechanism of Action of Whey Proteins**

Whey acts as an antioxidant and detoxifies, and this is due to its participation in the synthesis of glutathione (GSH), which is an intracellular antioxidant. It is the glial endogenous antioxidant produced in cells that allows protection of RNA, DNA and proteins through a redox cycle, from GSH (reduced form) to GSSH (oxidized form) (Bayford, 2010). Whey is rich in cysteine which combines with glutamate and glycine to form glutathione. GSH contains a thiol (sulphydryl) group that serves as an active reducing agent in the prevention of oxidation and tissue damage. Through direct conjugation, it detoxifies endogenous and exogenous toxins including toxic metals, petroleum distillates, lipid peroxides, bilirubin and prostaglandins. Riboflavin, niacinimide, and glutathione reductase are essential cofactors in glutathione reduction (Marz, 2010).

As a detoxifying agent, glutathione peroxidase (GSHPx), which is derived from selenium and cysteine, is an endogenous enzyme that has the ability to convert lipid peroxides into less harmful hydroxy acids.

Studies have shown that lactoferrin has the ability to activate natural killer cells (NKC<sub>s</sub>) and neutrophils (Nishiya, K., Horwitz, D. A., 1982).

Lactoferrin has antiviral, antifungal and antibacterial effects. It has the greatest impact on organisms that require iron to reproduce, as this protein has the ability to bind iron (Shash, 2000).

According to a study with rats, it was concluded that lactoferrin has an anti-inflammatory effect by regulating the levels of tumor factors (TNF) and interleukin 6 (IL-6) (Machinicki, M., Zimecki, M., Zagulski, T., 1993).

It has the ability to release the outer membrane of gram-negative bacteria, thus having an antibacterial effect (Tomita, 2002).

Whey has recently been used as a supplement to lower blood pressure, since antihypertensive peptides have been isolated from the primary sequence of bovine  $\beta$ -lactoglobulin (Mullally et al 1996). In animal studies,  $\beta$ -lactoglobulin has been shown to inhibit cholesterol absorption by altering its solubility in the intestine. Whey as a functional food. Therapeutic application of whey.

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

Whey proteins are high quality proteins and with their high percentage of branched chain amino acids (BCAA- branched chain amino acids), they have been popular in the fitness industry for a long time as muscle building supplements. But research shows that they may have much wider applications as functional foods for cancer, hepatitis B, cardiovascular disease, osteoporosis and chronic stress (Bounous, 2000).

Almost all whey proteins possess bioactivity per se and are used as ingredients in many commercial products such as health foods. Bioactive peptides are generated in vivo during digestion in the gastrointestinal tract and in vitro by milk proteases or enzymes from starter cultures.

Whey proteins are thought to act therapeutically against diseases associated with oxidative stress (Balbis et al, 2009), as cysteine-rich undenatured protein isolates increase glutathione levels by providing precursors needed for glutathione synthesis.

Whey proteins contribute to reducing the level of transaminases in the blood, especially alanine transaminase. An increased level of transaminases in the serum is an indicator of liver disease.

### **6.3 Whey Production**

Whey is the liquid that remains after the precipitation and removal of casein during cheese making. This by-product represents about 85-90% of the total volume of milk and contains 55% of milk components. Liquid whey can be used to produce a variety of mild and fermented beverages. By fermenting lactose, it can be used for the production of other products: alcohol, lactic acid or for the production of the antibiotic penicillin, vitamins B2 (riboflavin) and B12. Most often, liquid whey is used for feeding livestock or for the production of powdered fodder.

Water from whey can be removed by concentration procedures (classical evaporation or membrane concentration procedure), reverse osmosis and ultrafiltration. The higher amount of whey minerals in food products can cause grittiness, clumping and a sour salty taste, especially in sour whey because it contains a higher amount of calcium than mild whey. Sour whey also has a large amount of lactic acid, which causes technological problems as well as the appearance of lumps. For these reasons, mild whey is used in processing.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

Regardless of the type of processing and the length of the processing, the cheese dust must first be removed from the whey, by a filtration or clarification procedure, and then the fat must be removed by centrifugal separation. The separated milk fat can be used in the production of cheese, various dairy desserts and for the production of whey butter (Presilski, 2004).

### ***Cheese Whey***

Most of the whey (92%) is obtained during the production of different types of cheese. First, the milk is standardized to a fat content between 2.5% (40% fat in cheese) and 3.5% (full-fat cheese). Standardization and pasteurization are performed with a pasteurizer and separator. The reduction of the fat content of cow's milk containing more than 4% fat is achieved by adding cream. Immediately afterwards, the standardized milk passes through the pasteurizer for the inactivation of bacteria obtained by contamination. The milk is subsequently cooled to a temperature of 30°C and inoculated with starter (a culture of lactic acid bacteria) and rennet, a mixture of the enzymes chymosin and pepsin naturally found in the intestines of young calves. The starter gives the desired characteristic properties to the cheese, and the rennet (or its substitute) produces gelation of the casein, incorporating fat globules from the milk into the gel. This milky gel forms in about 30 minutes at 30°C, and then it is cut into cubes. They will precipitate into the cream, leaving the whey as a supernatant (de Wit, 2001).

### ***Casein Whey***

Casein and caseinates are produced by acidification of pasteurized skimmed milk with a culture of lactic bacteria at 25°C or with acids such as hydrochloric acid or sulfuric acid at 45°C. Casein will precipitate around pH 4.6 and is separated from the resulting liquid by centrifugation or by decantation followed by washing (de Wit, 2001). After separation, the casein curd is dried to obtain casein powder suitable for various industrial applications. Caseinates are then produced by neutralizing casein with alkaline substances such as sodium or calcium hydroxide, resulting in products with improved solubility.

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

### **7. OBJECTIVES OF THE RESEARCH**

The purpose of this research is to investigate the healing power of whey and whey proteins for humans (for example its effect on liver diseases), by administering to pigs that have the same organic and metabolic function as humans. In this master's thesis, the transaminases ALT, AST and  $\gamma$ -GT in the blood of pigs, which are the biggest indicators of liver damage, as well as some minerals (Ca, K, Fe and P) and total protein and albumen in the blood of piglets were investigated. and in the whey. The purpose of this research is to see the influence of whey on transaminases, that is, on liver function. Increased levels of alanine aminotransferase-ALT and aspartate aminotransferase-AST indicate liver damage, so it is assumed that whey proteins contribute to the reduction of transaminase levels. For this purpose, two groups of piglets were administered with different amounts of high protein whey.

- Examination of transaminases ALT, AST and  $\gamma$ -GT (in vivo) in blood serum of piglets administered with whey during 45 days,
- Examination of the transaminases ALT, AST and  $\gamma$ -GT (in vitro) in normal N and pathological P (HUMATROL P and N) control serum with the presence of whey,
- Calculation of the inhibitory activity of whey on the examined enzymes.
- Examination of the composition of whey (minerals: Ca, K, Fe and P, total proteins and albumins) and their impact on diseases
- The influence of whey as an inhibitor on other enzymes
- Examination of the significance of transaminases as indicators of diseases,

#### **7.1 Material And Methods Of Work**

The research was conducted on the pig farm of ZK "Pelagonija". The piglets were administered protein-rich whey from mixed cheese and cheese from the "Ideal Shipka" dairy - Bitola.

Piglets were divided into three groups. One was a control group, which was fed only concentrate. The second group of piglets was administered a defined amount of whey in addition to the concentrate, and the third group of piglets was administered ad libidum.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

Every two weeks the amount of whey given was increased. In the following text, the following abbreviations will be used:

**Table 6.** Amount of whey added to the diet of piglets during 45 days

Days	0-15	15-30	30-45
<b>C group</b>	0	0	0
<b>B group</b>	~2L	~5L	~10L
<b>A group</b>	~10L	~15L	~20L

During the entire research, the weight of the piglets was monitored before and after administration with whey (Table 7).

**Table 7.** Weight of piglets before and after the research

	Controlled group	A group	B group
at the beginning of the research	~20 kg	~40 kg	~20 kg
at the end of the research	~50 kg	~70 kg	~50 kg

When measuring the weight of the piglets in both groups before and after the research, no change was observed compared to the control group. In relation to the group itself, from before the research and after the research, the weight of the piglets increased by 25% in the K group, 17.5% in the A group, and 25% in the B group.

All were followed for a period of 45 days, and every 15 days including day zero, blood was taken to examine the amount of transaminases in the serum, to see the in vivo change of ALT, AST and  $\gamma$ -GT (in vivo). The measurement of transaminases was performed by spectrophotometric methods in the biochemical laboratory "Bitolska laboratory" - Bitola. Spectrophotometric, photometric - colorimetric and nephelometric methods were also applied to prove the amount of certain components present in the whey composition.

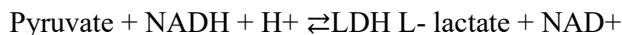
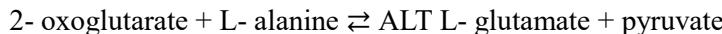
In vitro studies were also performed with whey to which HUMATROL P (serum with pathological values) and N (serum with normal values) an animal serum-based control serum was added, to see the inhibitory power of whey on transaminases ALT and AST.

All parameters were determined spectrophotometrically, on a Screen Master spectrophotometer intended for clinical chemistry.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

### ***Determination of Alanine Aminotransferase (ALT) - Spectrophotometric Test***

A kinetic method is used for the determination of ALT activity, according to the recommendations of the International Federation of Clinical Chemistry. The principle of the reaction is as follows (Schumann, G. et al, 2002):

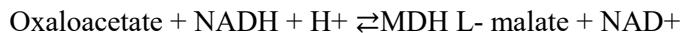
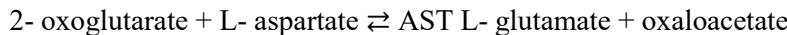


The reagent consists of two parts, buffer/enzyme reagent and substrate, which are mixed in a ratio of 1:4 and the absorbance is measured at a wavelength of 365 nm, 334 nm or 340 nm.

If working at 37 oC, add 50 µl of serum to 500 µl of the prepared working reagent, if working at a lower temperature, add 100 µl of serum.

### ***Determination of Aspartate Aminotransferase AST - Spectrophotometric Test***

A kinetic method is used for the determination of AST activity, according to the recommendations of the International Federation of Clinical Chemistry. The principle of the reaction is as follows (Schumann, G., et al, 2002):



The reagent consists of two parts, buffer/enzyme reagent and substrate, which are mixed in a 1:4 ratio, the working reagent thus prepared is stable for 4 weeks at a temperature of 2 oC to 8 oC and 5 days at a temperature of 15-25 oC.

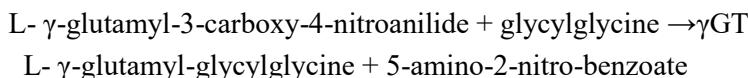
The absorbance is measured at a wavelength of 365 nm, 340 nm or 334 nm, the optical path being 1 cm. The temperature can be 25 oC, 30 oC or 37 oC. During the measurement, care must be taken to ensure that there is no air, that is, that no bubbles are created because they reduce the absorbance.

If working at 37 oC, add 50 µl of serum to 500 µl of the prepared working reagent, if working at a lower temperature, add 100 µl of serum.

**CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL  
NUTRITION**

***Determination Of  $\Gamma$ gt - Gamma Glutamyl Transferase -  
Colorimetric Test***

The activity of  $\gamma$ GT is determined by a colorimetric kinetic method. This method is standardized against the recommended IFCC method. The reaction principle is as follows (Schumann, G., et al 2010):



The reagent consists of two parts, a buffer and a substrate. The reagent must be heated to the desired temperature (25 oC, 30 oC or 37 oC) and that temperature maintained constant throughout the test. The working reagent is prepared 4:1, ie 4 parts of the buffer and 1 part of the substrate. Absorbance is measured at a wavelength of 400-420 nm. Mix 600  $\mu$ l of the working reagent with 60  $\mu$ l of the sample.

***Determination of iron - photometric colorimetric iron test with  
fat clearance factor (LCF)***

Iron (III) reacts with chromazurol B (CAB) and cetyltrimethylammonium bromide (CTMA) and a colored complex is formed with an absorbance maximum at 623 nm. The intensity of the color is directly proportional to the concentration of iron in the sample (Garcic, 1979)

Care should be taken to avoid reagent contamination. Fatty samples cause falsely high results, so this reagent has a fat clearance factor (LCF).

Mix 500  $\mu$ l of reagent with 25  $\mu$ l of the sample and incubate for 15 minutes at room temperature. The absorbance of the sample is measured within 60 minutes against a blank for the entire run and measured at a wavelength of 623 nm.

This test is linear up to 500  $\mu$ g/dl or 89.5  $\mu$ mol/l. Great care should be taken when performing this test because the test is very sensitive, disposable materials are used and the distilled water must not contain iron.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

### ***Determination of Calcium - Photometric Method***

Calcium ions react with o-cresolphthalein-complex in an alkaline environment and a violet-colored complex is formed. The absorbance of this complex is proportional to the concentration of calcium in the sample (Gitelman, 1967).

The reagent consists of two parts, from which the working reagent is prepared in a ratio of 1:1 and stands for 30 minutes at room temperature before use. The absorbance is measured at a wavelength of 570 nm (Hg 578 nm) and 546 nm, the optical path is 1 cm, the temperature should be 20-25 oC. The measurement is performed against a blank. 600 µl working reagent and 12 µl of the sample are mixed, after 5-30 minutes they are read.

### ***Determination of Phosphor - Photometric UV Test***

Phosphorus reacts with molybdate in a strongly acidic environment and a complex is formed. The absorbance of this complex is directly proportional to the concentration of phosphorus (Gamst O., Try, K., 1980).



The reagent is stable but contamination must be avoided. Absorbance is measured at a wavelength of 340 nm or 343 nm. Mix 600 µl of the reagent with 6 µl of the sample, incubate for at least 1 minute and within 60 minutes measure against the blank.

### ***Determination Of Potassium - Nephelometric Method (Endpoint)***

Potassium ions in a protein-free alkaline medium react with sodium tetraphenylboron (TPB-Na) to produce a dispersed colloidal suspension of potassium tetraphenylboron. The resulting turbidity is proportional to the concentration of potassium in the sample (Terri, A. E., Sesin, P.G, 1958).

The reagent consists of three parts. Reagent 1 is precipitate (trichloroacetic acid), reagent 2 is TPB-Na and reagent 3 is NaOH. The working reagent is prepared by mixing R2 and R3 in a 1:1 ratio. After it is prepared, it is preferable to let it stand for 15-30 minutes.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

The first part of the procedure is precipitation, that is, it is necessary to precipitate the proteins, therefore, mix 600 µl of reagent 1 with 60 µl of the sample, centrifuge for 10 minutes at 4000 rpm and then separate the clean supernatant.

The second part of the procedure is colorimetry. Mix 600 µl of the working solution reagent with 60 µl of the supernatant, incubate for at least 5 minutes and measure the absorbance at a wavelength of 578 nm against a blank sample.

### ***Determination Of Albumen - Photometric Colorimetric Test For Albumen With BCG-Method***

Bromocresol green in citrate buffer forms a colored complex with albumin. The absorbance of this complex is proportional to the concentration of albumen in the sample (Rodkey, 1965, Doumas, B., et al, 1971).

The absorbance is measured at a wavelength of 578 nm, the measurement is performed against a blank sample. Mix 600 µl of reagent with 6 µl of the sample and incubate for 5 minutes.

### ***Determination of Total Proteins - Colorimetric Photometric Method (Biuret Method)***

Copper ions from proteins and peptides with an alkaline environment form a violet colored complex. The absorbance of this complex is proportional to the concentration of proteins in the sample (Weichsebaum, 1946, Josephson, B., Gyllenswärd, C., 1957).

Absorbance is measured at a wavelength of 520-580 nm and the measurement is performed against a blank. Mix 600 µl of reagent with 12 µl of the sample and incubate for 10 minutes.

### ***Statistical Analysis of The Results By Analysis Of Variance-ANOVA***

In scientific-research work in agriculture, animal husbandry, veterinary medicine and more widely in biological sciences, comparisons of multiple modalities of one factor are carried out. The factor under investigation has at least three modalities (variants).

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

This is the case in our research where the factor is whey and we have three modalities, that is, three groups of piglets. The null hypothesis ( $H_0$ ) is that  $\bar{x}_1 = \bar{x}_2$ ;  $\bar{x}_1 = \bar{x}_3$ ;  $\bar{x}_2 = \bar{x}_3$  (Ott, R. L., Longnecker, M., 2001). If the null hypothesis is rejected, in order to confirm exactly which groups have a significant difference, further analysis is performed, in our case Tukey's HSD Post-hoc test. HSD is calculated using the formula:

$$HSD = q^*$$

q-table value

n the number of values used in the calculations

MSwithin- or variance, represents the square of the standard deviation, i.e. the average of the squared deviations of the variances from the mean value.

## 8. RESULTS

### *Analysis of the Constituent Components of Whey*

The composition of the whey was also examined, namely the mineral composition (Ca, P, K and Fe) and total proteins and albumen, to see if there is a relationship between some of these constituents of the applied whey and the activity of the enzymes.

**Table 8.** Amount of Ca in whey

Ordinal Number Of Trial Ca/ (Mmol/L)	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
1	7,58	6,12	8,91	6,22	7,31
2	8,22	6,89	8,93	6,14	7,22
3	8,28	6,12	9,88	6,28	7,15
4	7,53	4,78	8,78	6,13	7,11
5	7,58	6,19	9,15	6,2	7,11
6	7,49	5,42	9,54	6,42	7,11
7	8,3	5,78	8,77	6,16	7,19
8	7,59	5,56	12,5	6,14	7,13
9	8,12	5,75	9,24	6,14	7,2
10	8,01	5,81	8,99	6,22	7,25
$\bar{X}$	7,87	5,842	9,469	6,205	7,178
SD±	0,34	0,55	1,12	0,08	0,06

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

The third whey is the richest in Ca, around 9.5 mmol/l, while the second one contains at least 5.8 mmol/l Ca (Table 8). The second whey contains the most P, 9.4 mmol/l, and the others have approximately the same amount (Table 8). All 5 types of whey are rich in K and there is not much difference between them, the highest level of K has C4 26.6 mmol/l (Table 9). Fe is present in very small amounts compared to other minerals, the first whey contains the most 11.7  $\mu$ mol/l, while the others have about 9  $\mu$ mol/l (Table 10). It is important to note that the first two samples of whey are obtained from cheese, while the rest are from mixed cheese. These differences in mineral composition may be attributed to variations in cheese-making processes and raw milk characteristics. The type of cheese and processing conditions can significantly influence mineral retention in whey. Consequently, the nutritional value of whey may vary depending on its source and method of production.

**Table 9.** Amount of P in whey

Ordinal Number Of Trial P/ (Mmol/L)	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
1	7,16	8,12	6,28	7,75	8,21
2	7,78	11,09	10,99	7,85	8,22
3	6,76	4,89	7,25	7,93	8,2
4	7,15	9,73	8,91	7,65	8,23
5	6,84	8,79	7,33	7,83	8,25
6	6,95	11,5	7,58	7,8	8,2
7	7,04	11,11	7,62	7,62	8,2
8	7,23	9,02	7,49	7,71	8,15
9	6,75	9,49	8,05	7,8	8
10	6,9	10,21	9,15	7,75	8,2
$\bar{X}$	7,056	9,395	8,065	7,769	8,186
SD $\pm$	0,30	1,92	1,31	0,09	0,07

From Table 8, it can be seen that in C5 the deviation from the mean value of Ca is the smallest ( $SD\pm=0.06$ ), and the largest is in C3.

**CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

**Table 10.** Amount of K in whey

Ordinal Number Of Trial K/ (Mmol/L)	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
1	26,2	27	24,75	27	25
2	22,5	26,3	28,5	24,9	25,45
3	21,75	26,3	26	26,8	25,66
4	24,5	24,8	25,9	27,7	25,75
5	23,5	26	23,8	26,7	26,05
6	26,19	26,1	25,3	26,3	26,15
7	22,49	22,3	26,8	27,3	24,9
8	23,83	24,8	25,4	26,9	26,05
9	25,31	25,3	26,3	25,4	26,1
10	24,15	24,5	27	27	25,8
Ȑ	24,042	25,34	25,975	26,6	25,691
SD±	1,54	1,33	1,30	0,85	0,44

Whey is rich in proteins, it can be seen from table 12 and 13. The highest content of total proteins is the first whey obtained during the production of cheese, it contains 17.6 g/L, and the least contains C4 9.5 g/L obtained during the production of mixed cheese. Normally, C2 has the highest albumen content, 2.36 g/L, and C4 has the lowest content, 1.34 g/L.

These variations in protein content reflect differences in processing conditions and the type of cheese produced. Whey proteins such as albumins and globulins are known for their high biological value and digestibility. Higher protein concentrations enhance the nutritional and functional properties of whey in food applications. In addition, protein composition may influence the bioactive potential of whey, including its antioxidant and immune-supporting effects. Therefore, whey derived from cheese production can represent a valuable source of high-quality proteins.

**CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

**Table 11.** Amount of Fe in whey

Ordinal Number Of Trial Fe/	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
1	10,2	10,12	10,4	12	10,1
2	11,3	10,1	10,3	9	10,4
3	11,4	9,3	10,2	11,3	10,1
4	12,5	8,4	9,9	9,5	10,15
5	12,6	5,1	9,9	9,4	10,2
6	13	10,2	10,1	9,4	10,1
7	11,2	10,3	9,8	9,5	9,41
8	10,9	9,5	9,8	9,9	9,43
9	12,5	9,5	9,7	10,2	9,9
10	11,8	9,5	9,8	10,3	10,2
$\bar{X}$	11,74	9,202	9,99	10,05	9,999
SD $\pm$	0,89	1,54	0,24	0,94	0,32

From Table 12, it can be seen that the smallest deviation from the mean value for total proteins is in C 4 (SD $\pm$  =1.17), and the largest is in C1 (SD $\pm$ =3.94).

**Table 12.** Amount of total protein in whey

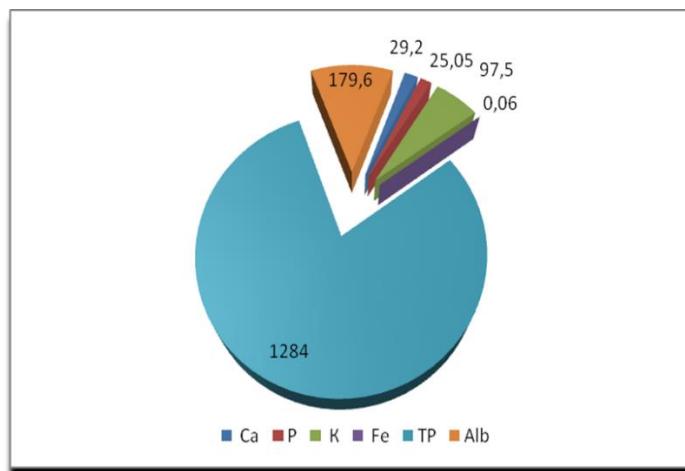
ordinal number of trial TP/ (g/L)	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
1	14	12	12	11	13
2	18	13	13	11	12
3	17	12	15	8	14
4	27	15	12	8	12
5	19	12	10	9	12
6	18	18	9	9	14
7	18	16	10	9	10
8	12	12	10	10	11
9	15	15	9	11	13
10	18	12	10	9	13
$\bar{X}$	17,6	13,7	11	9,5	12,4
SD $\pm$	3,97	2,16	1,94	1,17	1,26

**CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

**Table 13.** Amount of albumen in whey

Ordinal Number Of Trial Alb/ (G/L)	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
1	2	1,5	1,4	2	1,9
2	2,2	2	1,5	1,3	1,8
3	4,7	1,3	1,6	1,1	2
4	3	2,1	2	1,3	2,1
5	2,5	1,4	2,2	1,5	2,3
6	2,1	2,3	1,3	1,1	2
7	1,1	2,5	1,1	1,2	2,1
8	2	1,1	1,3	1,1	1,9
9	1,8	1,4	1,5	1,3	1,8
10	2,2	2	1,4	1,5	2
ȐX	2,36	1,76	1,53	1,34	1,99
SD±	0,95	0,47	0,33	0,27	0,15

If all the examined ingredients, that is, their average values are converted into mg/dL, the representation of each of them in the whey can best be seen (Graph 5). Proteins are the most abundant, K is the most concentrated of the minerals, and Fe has the lowest content.



**Figure 5.** Composition of the applied whey

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

### ***ALT Assay (In Vivo) in Whey-Fed Piglets***

On the first day, before starting administration with whey, blood was taken from the three groups of piglets. As expected between them, there is no difference in ALT values according to the ANOVA method.

By processing the data from the serum for ALT, with the ANOVA method, it was obtained that between the three groups of pigs there are significant differences in the values at the 15th and 45th day ( $F>F_{crit}$ ,  $p<0.05$ ). By further processing the data with Tukey's HSD Post-hoc test, it was obtained that on the 15th day there is a difference between groups A and B, and on the 45th day there is a difference between the K and A samples. On the 30th day, there is no significant difference between the ALT values for the three groups.

Table 14 shows all the results for ALT obtained during the research, and in graph 1 they are shown graphically and the comparison between them can be seen. The smallest deviation, that is, the lowest value of  $SD\pm$  (4.5) and  $CV$  (4.4) has group A on the 15th day, the highest values for  $SD\pm$  (47.6) have the control group on day 45, and for  $CV$  (35.7) group K on the 30th day.

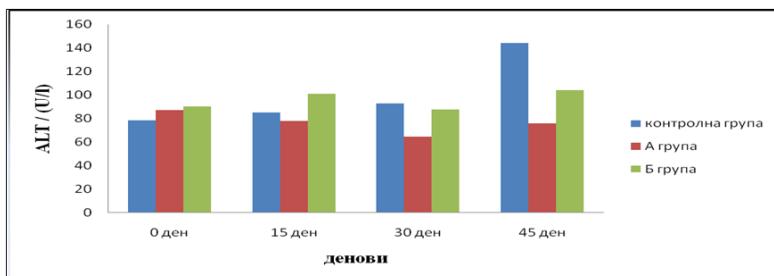
From table 14 it can be observed that on day zero the  $SD\pm$  and  $CV$  in the three groups of piglets are approximately the same.

On the 15th day,  $SD\pm$  and  $CV$  in groups A and B fed with whey was significantly reduced compared to the zero day, while in the control group they were increased. Group B, which is fed with a controlled amount of whey, has the lowest value, which is 2L per day for 7 piglets. But the mean value of ALT is the lowest in group A which is fed ad libitum.

On day 30,  $SD\pm$  and  $CV$  were significantly increased in the control group compared to day zero, while in groups A and B they continued to decrease, especially in piglets administered ad libidum. Which means that the higher amount of whey in their food has a positive effect. Also on day 45 there is a very significant difference in  $SD\pm$  and  $CV$  values compared to day zero, especially in the control group and group A. The values of K are drastically increased, while group A has the lowest values for  $\bar{x}$ ,  $SD\pm$  and  $CV$ , from which it can be concluded that feeding with a larger amount of whey has a positive effect.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

In group B, which was administered with a controlled amount of whey about 10L per day for 7 piglets, a minimal decrease compared to the zero day was observed, but significantly lower values compared to the control group. Chart 1 best shows the difference in mean values between groups.



**Figure 6.** Comparison of ALT between the three groups of piglets

### *Assay of AST (In Vivo) in Whey-Fed Piglets*

The same procedure was repeated for AST, that is, the value of serum AST was examined for the control group, group A and group B, on the zero day before starting administration with whey, on the 15th day, the 30th day and the 45th day. The data were statistically processed with the ANOVA method and Tukey's HSD Post-hoc test. The obtained data show that on day 45, the differences between the control group and group A administered ad libitum are statistically significant at the  $p<0.05$  level.

Table 15 and graph 3 show the AST values for day zero, day 15, day 30 and day 45, showing the difference between the three groups. From this table it can be seen that group K has the smallest deviation (5.2) and coefficient of variation (9.1) on day 30, and group B has the highest values on day 45 ( $SD\pm=36.8$  and  $CV=42.1$ ).

On day zero, CV and  $SD\pm$  in groups A and B have a high value, but then they decrease. On the 15th day  $SD\pm$  and CV in group B fed with whey is significantly reduced compared to day zero, in A there is no significant change, while in the control group they are increased. Group B, which is fed with a controlled amount of whey, has the lowest value, which is 2L per day for 7 piglets. But the mean value of AST is the lowest in group A which is fed ad libidum.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

On the 30th day, the mean values in the three groups are approximately the same, but the lowest is in group A. SD $\pm$  and CV are lowest in the control group, while in group B they continue to decrease.

On the 45th day, there is a significant decrease in values in group A, it has the lowest mean value and CV and SD $\pm$ , while in the control group they are the highest. Which means that the higher amount of whey in their food has a positive effect. Graph 3 best shows the difference in mean values between the groups.

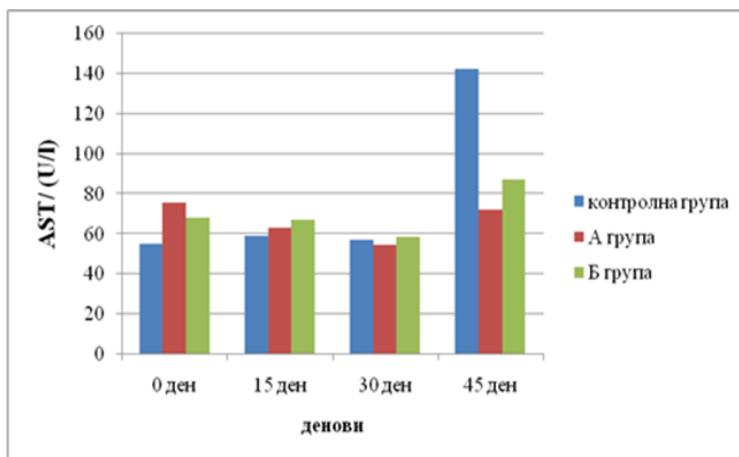


Figure 7. Comparison of AST between the three groups of piglets

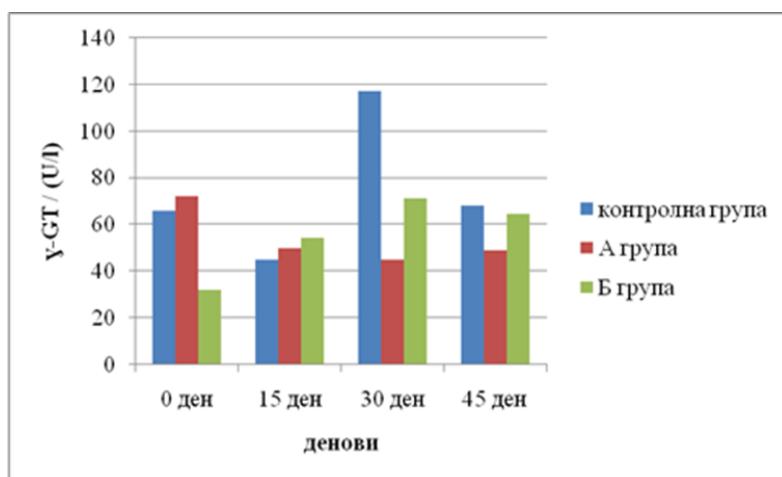
### *Examination of $\gamma$ -GT (In Vivo) in Whey-Fed Piglets*

The level of  $\gamma$ -GT in the blood of piglets was examined on day zero, day 15, day 30 and day 45. With the statistical processing of the data, it was obtained that there is a significant difference between the groups at the  $\alpha = 0.05$  level, on the zero and the 30th day. These data are given in table 16 and graph 4.

For the zero day, it was found that there is a significant difference between group B with K and A (attachment). On the 15th and 45th day, there is no significant difference in the mean values at the  $p < 0.05$  level (appendix). On the 30th day, with the statistical processing of the data, it was obtained that there is a significant difference at the  $p < 0.05$  level, between the control group and groups A and B. From this it can be concluded that by applying 5-15 L of whey daily in the diet, it has a positive effect on  $\gamma$ -GT.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

In Table 16 you can see all the results for  $\gamma$ -GT obtained during the research. On the zero day, the values for  $SD \pm$  and CV in the three groups differ from each other, the lowest values are in group A and the mean value is the smallest in group B. On the 15th day, only a decrease in the mean value is observed in group A compared to the zero day. On the 30th day, the mean value in the K group was significantly increased compared to the zero day, and it has the highest value among the three groups. Although  $\bar{x}$  is the lowest for group A, the  $SD \pm$  and CV values are the lowest for group B. On day 45, group A administered ad libidum has the lowest  $\bar{x}$  both in relation to day zero and in relation to other groups of piglets at the end of the research, i.e. day 45. From this it can be concluded that the application of a greater amount of whey in the diet affects the reduction of the amount of  $\gamma$ -GT present in the blood serum of piglets.



**Figure 8.** Comparison of  $\gamma$ -GT between the three groups of piglets

### ***Monitoring of ALT, AST and $\gamma$ -GT In Vitro***

In addition to in vivo, the in vitro influence of whey on transaminases was also monitored. By inhibiting pathological and normal serum (HUMATROL P and N) with whey, the following results were obtained, shown in table 11 and 12.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

An analysis of ALT, AST and  $\gamma$ -GT in pathological serum was performed, namely 3 sample samples using 3 different serums and the largest positive deviations from the mean value for ALT of 10.01U/L and the smallest for  $\gamma$ -GT and that of 2.03 U/L which speaks of high accuracy of distribution of values around the mean value.

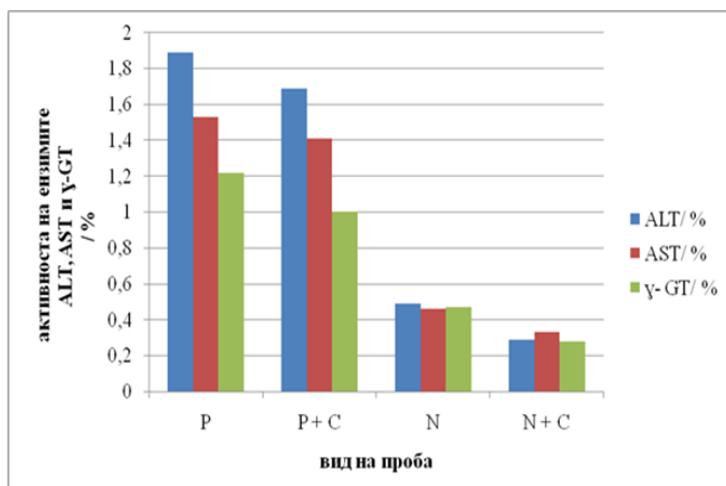
### ***Calculation of the Inhibitory Activity of Whey on Enzymes***

The percentage of whey inhibition on transaminases is calculated mathematically according to the formula (Kaiser, C., van der Mewe, R., Bekker, T.F., Labuschagne, N., 2007):

$$\% \text{ of inhibition} = [(\text{normal activity} - \text{inhibited activity}) / (\text{normal activity})] * 100\%$$

It was found that whey inhibits ALT 10.71%, AST 8.51% and  $\gamma$ -GT 18.16% in pathological serum. In serum with normal values, whey inhibits ALT 39.33%, AST 29.08% and  $\gamma$ -GT 39.59%.

Whey acts as an inhibitor on the activity of ALT, AST and  $\gamma$ -GT, this can also be seen from table 13 where their activity is expressed in percentages, it is noted that in both pathological and normal serum the activity decreases by about 0, 2%.



**Figure 9.** The enzyme activity in P and N, with and without influence of whey

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

### **9. DISCUSSION**

The tests done on ALT, AST and  $\gamma$ -GT enzymes in pigs administered with a fixed and undetermined (ad libidum) amount of whey in vivo, as well as tests done in vitro (using pathological and normal serum) showed the great influence of whey and its importance in piglet nutrition. Our results from this research show that the application of whey in the diet of piglets leads to a reduction of transaminases, both in live and in vitro tests, and we prove that whey is an inhibitor, i.e. reduces the enzymatic activity of ALT, AST and  $\gamma$ -GT enzymes.

Transaminases are the main indicators of liver diseases. That's why serial hepatic researches have been done during hypoxia in newborn piglets, for ALT, AST and LDH in order to predict liver injury because little is known about the correlation of the values of the different enzymes that are indicators of normal and injured liver (Mathias Karlsson et al, 2009). Alanine aminotransferase (ALT) is an important enzyme that is found mostly in the liver. Its increased activity usually occurs due to leakage from damaged liver cells. Aspartate aminotransferase (AST) is similar to ALT and its activity is related to the liver, but it can also be an indicator of diseases of other organs.

Since it is assumed in the literature that whey proteins contribute to the reduction of transaminase levels, we used whey as a supplement in the diet of piglets. It is a by-product in the production of cheese, which was previously considered waste and is a very cheap source of fodder.

Pigs are very similar to humans, that is, organically and metabolically they have the same function, therefore we can adequately examine the healing power of whey on humans by administering whey to pigs.

In this study, the changes of ALT, AST and  $\gamma$ -GT in vivo were monitored to see the influence of whey on enzyme activity. For this purpose, 2 groups of 7 piglets were administered with different amounts of whey and those values were compared with a control group that was fed only with concentrate. In accordance with our research is the research of Chitapanarux et al (2009) according to which by giving a daily dose of 20 g of whey protein isolates for a period of 12 weeks to patients with fatty liver, there was a significant reduction of ALT and AST. In our research, a decrease in transaminases was observed after 45 days of whey consumption in different amounts.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

The value of transaminases of the control group that was fed only with concentrate was in a constant increase, while in groups A and B a constant decrease in enzyme activity was observed. Group A, which was administered ad libidum, had the greatest reduction after 45 days compared to the control group (Tables 8, 9 and 10).

Cheeke and Stangel (1973) in their study administered 5 groups of piglets with different diets using whey and lactose. They concluded that the growth rate and productivity of piglets fed a diet containing 10% whey and 20% alfalfa (a source of vegetable fiber) was lower than those groups of piglets that were administered only one of these two, leading to the conclusion that there may be antagonism between these two ingredients. In our case, the weight of the examined piglets was measured at the beginning and after the end of the research, and no significant change in weight compared to the control group was observed (Table 7), but before the research and after the research, the weight of the piglets increased and that in K group by 25%, A group by 17.5% and B group by 25%.

Research has been done by administering mixed forage to pigs with a higher level of lysine in the diet and an improvement in some % of carcass meat in the area of the Longissimus dorsi, as well as a reduction in average fat thickness, was obtained. A consequence of this research was to determine the influence of different levels of lysine from compound feeds on improved performance of fattening pigs (Marin, M., Dragotiou, D., Pana, C., Pogurschi, E., 2003).

The influence of whey on the activity of ALT, AST and  $\gamma$ -GT was also investigated in vitro, using control sera with normal and pathological values. Our tests showed that whey acts as an inhibitor on the activity of these enzymes (Table 13). Since we do not know exactly which active ingredients have an inhibitory effect on transaminases, the composition of the used whey was investigated, if it is possible to find a relationship between the composition and the inhibition. From the examination it was concluded that whey has the highest percentage of proteins (Graph 5). Of the minerals, potassium is the most abundant, followed by calcium, while iron is found in a very small percentage.

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

Hamad et al. (2011) in their research determined that with oral administration of whey proteins up to results in a decrease in ALT and AST values in rats with fatty liver. In our research, whey is rich in Ca, the third sample has the highest mean value of 9.5 mmol/l (Table 14). According to Siddiqui et al. (2008) intake of higher amounts of whey protein, Ca, and vitamin D resulted in reduced fat accumulation and proportionally increased insulin receptor expression, regardless of the level of calories from fat or sucrose. From our tests, it can be seen that the whey contains the most proteins as expected, especially the first two samples obtained during the production of 12 g/L cheese (Table 18). In research by Wu et al. (1998) concluded that combining whey and potassium improves the condition of heart diseases. The whey used contains a high amount of K, the most is C4 26.6 (Table 16).

The results of this research do not provide an answer as to which active ingredients in the whey inhibit the enzyme activity. It is assumed that some amino acids are responsible for this, but this leaves room for further research, because the control of liver diseases and the application of whey is a very important issue.

## **CONCLUSION**

The aim of the whole research is to see the influence of whey on transaminases in piglets. From the rejected results it can be concluded that:

- Whey significantly reduces the activity of ALT and AST in piglets administered ad libitum during 45 days compared to the control group;
- Whey significantly reduces  $\gamma$ -GT activity up to 30 days in piglets administered in a controlled amount and ad libitum;
- Whey exerts inhibition during in vitro testing of sera with normal and pathological values for ALT, AST and  $\gamma$ -GT;
- High values of transaminases are indicators of liver diseases, which means that whey helps in the treatment of these diseases by reducing their serum activity;
- Proteins are the most abundant of the tested ingredients in whey, and potassium is the most abundant of the minerals.

## *CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION*

The results of these researches will give a modest contribution to the expansion of scientific knowledge in applied biochemistry, especially in clinical enzymology in animals and in humans that have the same organic and metabolic functions. Enzymes as an important factor for many metabolic processes that take place in the living organism will encourage further research to determine which active ingredients from whey are responsible for the reduction of serum transaminase activity.

**CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL  
NUTRITION**

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*CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL  
NUTRITION*

**CHAPTER 3**  
**MONOGASTRIC NUTRITION AND WATER IN**  
**ANIMAL NUTRITION**

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# CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

## INTRODUCTION

Monogastric nutrition encompasses the dietary and metabolic processes in animals with a single-chambered stomach, including species like pigs, poultry, rabbits, and humans. Unlike ruminants, these animals possess a relatively straightforward digestive system, relying heavily on enzymatic breakdown in the stomach and small intestine for efficient nutrient extraction from feeds such as grains, cereals, and protein sources. This means that they have to rely on efficient absorption of nutrients and digestion to obtain the energy and nutrients they need to thrive. This chapter introduces key elements of monogastric nutrition, focusing on the metabolism of major nutrients (carbohydrates, proteins, and fats), nutrient absorption, energy metabolism, and protein synthesis, while also addressing the critical role of water as a macronutrient essential for health and productivity.

### 1. METABOLISM OF MAJOR NUTRIENTS

#### *Carbohydrates*

The main sources of carbohydrates for monogastric animals are grains and cereals. Carbohydrates are broken down into glucose, a simple sugar, by digestive enzymes in the mouth, stomach, and small intestine. Glucose is then absorbed into the bloodstream and transported to the liver, where it is used for energy or stored as glycogen.

Monosaccharides like glucose are absorbed primarily in the duodenum and jejunum of the small intestine. The intestinal lining features villi and microvilli, vastly increasing surface area for absorption. Glucose uptake uses active transport via SGLT1 (sodium-glucose linked transporter), which moves glucose against its concentration gradient using sodium pumps (energy from ATP). Fructose uses facilitated diffusion via GLUT5. Once inside enterocytes, monosaccharides enter the bloodstream via GLUT2 transporters and travel through the portal vein to the liver.

#### *Proteins*

Proteins are composed of amino acids, which are essential building blocks for all tissues in the body. Monogastric animals obtain amino acids from plant and animal sources.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

Proteins are broken down into peptides by digestive enzymes in the stomach and small intestine. Peptides are then further broken down into amino acids, which are absorbed into the bloodstream and transported to the liver and other tissues.

### ***Fats***

Fats are broken down into fatty acids and monoglycerides by digestive enzymes in the small intestine. Fatty acids and monoglycerides are then absorbed into the bloodstream and transported to the liver, where they are used for energy or stored as body fat. Dietary fats, primarily triglycerides (triacylglycerols), along with smaller amounts of phospholipids, cholesterol, and fat-soluble vitamins, are hydrophobic and do not mix easily with the watery environment of the digestive tract. In monogastrics (pigs, poultry, rabbits, humans), fat digestion is minimal in the mouth and stomach—lingual/gastric lipases play a minor role (e.g., more in young animals for milk fats)—so the small intestine is the main site.

## **2. ABSORPTION OF NUTRIENTS**

The absorption of nutrients takes place in the small intestine. The small intestine is lined with villi, which are tiny finger-like projections that increase the surface area for absorption. The walls of the villi are made up of enterocytes, which are cells that absorb nutrients from the food that is passing through the small intestine.

### ***Metabolism of Nutrients***

Once nutrients have been absorbed into the bloodstream, they are transported to the liver and other tissues, where they are metabolized. Metabolism is the process by which nutrients are converted into energy and other substances that the body needs to function.

The metabolism of nutrients is a complex process that is essential for the survival of monogastric animals. By understanding how nutrients are digested, absorbed, and metabolized, we can better understand the nutritional needs of monogastric animals and develop better diets for them.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

Protein metabolism in monogastric animals is a complex process that involves digestion, absorption, and utilization of amino acids.

### ***Digestion***

In monogastric animals (pigs, poultry, rabbits, humans), protein digestion begins in the stomach, marking the first significant breakdown of dietary proteins into smaller units. This acidic environment is crucial, as it prepares proteins for further digestion in the small intestine.

The stomach's parietal cells in the gastric glands secrete hydrochloric acid (HCl), creating a highly acidic environment (pH 1.5–3.5). This low pH serves two main purposes:

### ***Denatures Proteins***

Native proteins are tightly folded into complex 3D structures (secondary, tertiary, quaternary) held by hydrogen bonds, ionic bonds, and hydrophobic interactions. Acid disrupts these weak bonds, causing the protein to unfold or "denature," exposing the internal peptide bonds (the links between amino acids in the polypeptide chain). Provides the optimal acidic environment for the next enzyme and kills many ingested pathogens (antibacterial effect). Without denaturation, peptide bonds would remain hidden and inaccessible to enzymes.

Chief cells in the gastric glands secrete pepsinogen, an inactive precursor (zymogen). This prevents the enzyme from digesting the stomach's own cells. In the acidic lumen, HCl cleaves a peptide fragment from pepsinogen, converting it to active pepsin. Pepsin is an endopeptidase, meaning it cleaves internal peptide bonds (not just at ends). It preferentially cuts bonds involving aromatic amino acids (e.g., phenylalanine, tyrosine, tryptophan), producing smaller peptides (oligopeptides) and some free amino acids. This gastric phase efficiently initiates protein breakdown, ensuring amino acids become available for later absorption and utilization in growth, repair, and metabolism across monogastric species. The result is a mixture of partially digested peptides (rather than individual amino acids) that move into the small intestine for complete breakdown. This gastric phase is efficient but limited only about 10–20% of protein digestion occurs here. The bulk happens in the small intestine with pancreatic and brush-border enzymes.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

In young monogastrics (e.g., piglets, chicks), gastric digestion is less developed at birth, relying more on milk-clotting enzymes like chymosin initially. This step is vital for making proteins digestible, ensuring amino acids become available for absorption and utilization in growth, repair, and metabolism.

### ***Absorption***

Peptides from the stomach enter the small intestine, where further digestion occurs. Pancreatic proteases, including trypsin, chymotrypsin, and elastase, cleave peptides into even smaller fragments, dipeptides and tripeptides. These small peptides are further broken down into individual amino acids by intestinal peptidases, such as aminopeptidase and carboxypeptidase. Amino acids are then absorbed into the bloodstream through the enterocytes, specialized cells lining the small intestine.

### ***Utilization***

Absorbed amino acids are transported to various tissues throughout the body, where they are used for various functions, including:

- Tissue synthesis and repair: Amino acids are essential building blocks for proteins, which are the structural components of cells and tissues. They are also involved in tissue repair and regeneration.
- Enzyme and hormone production: Amino acids are essential components of enzymes and hormones, which play crucial roles in various biological processes, such as digestion, metabolism, and cell signaling.
- Energy production: In the absence of carbohydrates or fats, amino acids can be converted into glucose through gluconeogenesis, a process that occurs primarily in the liver. Glucose can then be used for energy production.
- Nitrogen excretion: Excess amino acids are not stored in the body and are metabolized into waste products, primarily urea. Urea is transported to the kidneys and excreted in urine.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

### **3. METABOLISM**

Metabolism is referred to the sequence, or succession, of chemical reactions that take place in the living organism. Some of the reactions involve the degradation of complex compounds to simpler materials and are designated catabolic reactions, whereas other reactions involve the synthesis of more complex compounds from simpler substances and are designated anabolic reactions. Waste products arise as a result of metabolism and these have to be chemically transformed and ultimately excreted; the reactions necessary for such transformations form part of general metabolism. As a result of various catabolic reactions, energy is made available for mechanical work, transportation and anabolic activity such as the synthesis of carbohydrates, proteins and lipids. The starting points of metabolism are the substances absorbed after the digestion of food. For all practical purposes we may regard the end products of carbohydrate digestion in the simplestomached animal as glucose, together with very small amounts of galactose and fructose. These are absorbed into the portal blood and carried to the liver. Digestion of proteins results in the production of amino acids and small peptides, which are absorbed via the intestinal villi into the portal blood and are carried to the liver, where they join the amino acid pool. They may then be used for protein synthesis in situ or may pass into the systemic blood, where they join the amino acids produced as a result of tissue catabolism in providing the raw material for the synthesis of proteins and other biologically important nitrogenous compounds.

Amino acids in excess of this requirement are carried to the liver and broken down to ammonia and keto acids. The latter may be used for amino acid synthesis or the production of energy. Some of the ammonia may be used in amination, but the majority is converted into urea and either excreted in the urine or recycled in the saliva. Most dietary lipids enter the lacteals as chylomicrons, which enter the venous blood vessels via the thoracic duct. The chylomicrons are about 500 nm in diameter with a thin lipoprotein envelope. A very small proportion of dietary triacylglycerols may be hydrolysed to glycerol and lowmolecular-weight acids in the digestive tract and these are absorbed directly into the portal blood supply. Circulating chylomicrons are absorbed by the liver and the triacylglycerols are hydrolysed.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

The fatty acids so produced, along with free fatty acids absorbed from the blood by the liver, may be catabolised for energy production or used for the synthesis of triacylglycerols. These then reenter the blood supply in the form of lipoprotein and are carried to various organs and tissues, where they may be used for lipid synthesis, for energy production and for fatty acid synthesis. Fatty acids catabolised in excess of the liver's requirement for energy are changed to (L-) Betahydroxybutyrate and acetoacetate, which are transported to various tissues and used as sources of energy.

### **3.1 Protein Synthesis**

Proteins are synthesised from amino acids, which become available either from the end products of digestion or as the result of synthetic processes within the body. Direct amination may take place as in the case of alpha-ketoglutarate, which yields glutamate. The glutamate may undergo further amination to give glutamine but, more importantly, may undergo transamination reactions with various keto acids to give amino acids. Amino acids other than glutamate may undergo such transaminations to produce new amino acids. Thus, both alanine and glycine react with phosphohydroxypyruvate to give serine. Glutamate is the source material of proline, which contains a five-membered ring structure. The synthesis of proline takes place in two stages and requires energy in the form of reduced nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).

Amino acids may also be formed by the reaction of keto acids with ammonium salts or urea; arginine, as we have already seen, may be synthesised during the formation of urea. Not all amino acids are capable of being synthesised in the body, and others are not synthesised at sufficient speed to satisfy the needs of the body. Both these groups have to be supplied to the animal. Such amino acids are known as essential or indispensable amino acids. The words 'essential' and 'indispensable', as used here, imply not that other amino acids are not required for the wellbeing of the animal but simply that a supply of them in the diet is unnecessary. All of the 25 amino acids normally found in the body are physiological essentials; some 10 or 11 are dietary essentials.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

As would be expected, the actual list of essential amino acids differs from species to species. Amino acids are absorbed from the gut into the bloodstream by active transport and transferred to the cells. This requires a supply of energy, since the concentration of amino acids in the cell may be up to 100 times that in the blood and transfer into the cell has to take place against a very considerable concentration gradient. A continuous exchange takes place between the blood and cellular amino acids, but not between the free amino acids and those of the tissue proteins. The tissue proteins themselves undergo breakdown and resynthesis, but their stability varies between different tissues. For example, liver protein has a half-life of 7 days whereas collagen is so stable that it may be considered to be almost completely inert. The process of protein synthesis may be divided conveniently into four stages: activation of individual amino acids, initiation of peptide chain formation, chain elongation and chain termination.

### **3.2 Regulation of Protein Metabolism**

Protein metabolism is tightly regulated by various hormones, including:

- Insulin: Insulin promotes amino acid uptake into cells and inhibits protein breakdown.
- Glucagon: Glucagon stimulates protein breakdown to release amino acids for gluconeogenesis.
- Thyroid hormones: Thyroid hormones increase protein synthesis and breakdown.
- Cortisol: Cortisol promotes protein breakdown to release amino acids for energy production.

### **3.3 Factors affecting protein metabolism**

Several factors can affect protein metabolism in monogastric animals, including:

- Dietary protein intake: The amount and quality of protein in the diet significantly impact protein metabolism. A diet deficient in protein can lead to protein deficiency, while an excessive protein intake can put a strain on the kidneys and liver.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

- Age: Protein metabolism is more efficient in young animals than in older animals.
- Physiological state: Pregnancy, lactation, and growth increase the demand for amino acids,
- affecting protein metabolism.
- Health status: Diseases and infections can impair protein metabolism.

### **4. ENERGY METABOLISM**

Energy may be defined as the capacity to do work. There are various forms of energy, such as chemical, thermal, electrical and radiant, all of which are interconvertible by suitable means. For example, the radiant energy of the sun is used by green plants, via photosynthesis, to produce complex plant constituents, which are then stored. The plants are consumed by animals and the constituents broken down, releasing energy, which is used by animals for mechanical work, for transport, for maintaining the integrity of cell membranes, for the synthesis of body components and for providing heat under cold conditions. Since all forms of energy can be converted to heat, heat units have been used to represent the energy involved in metabolism. Traditionally, the basic unit used has been the thermochemical calorie (cal), based on the calorific value of benzoic acid as the reference standard. However, the calorie is too small a unit for routine use and the kilocalorie (1000 cal) or megacalorie (1 000 000 cal) is more commonly used in practice. However, the International Union of Nutritional Sciences and the National Committee of the International Union of Physiological Sciences have now suggested the joule (J) as the unit of energy for use in nutritional, metabolic and physiological studies. The joule is defined as 1 newton per metre, and  $4.184 \text{ J} = 1 \text{ cal}$ .

### **5. THE METABOLISM OF FAT**

The metabolism of fat is a complex process that involves the breakdown (catabolism) and synthesis (anabolism) of fats. Fat is stored in the body as triglycerides, which are molecules made up of three fatty acids attached to a glycerol backbone.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

### ***Catabolism of Fat***

The breakdown of fat into energy is called beta-oxidation. This process occurs in the mitochondria of cells and involves the removal of two-carbon units from the fatty acid chain, one at a time. Each two-carbon unit is converted into acetyl-CoA, which can then enter the Krebs cycle to produce energy. The Krebs cycle is a series of chemical reactions that takes place in the mitochondria of cells and produces energy in the form of ATP. Acetyl-CoA can also be used to synthesize ketones, which are an alternative source of energy for the body, especially during times of starvation or low carbohydrate intake.

### ***Anabolism of Fat***

The synthesis of fat from other molecules is called lipogenesis. This process occurs in the liver and adipose tissue. Lipogenesis can occur from glucose, amino acids, and acetyl-CoA.

### ***Digestion of Fats***

Dietary fats, primarily triglycerides, are broken down into smaller molecules, monoglycerides and fatty acids, by digestive enzymes in the small intestine. Bile acids, produced by the liver and stored in the gallbladder, emulsify fats, increasing their surface area for enzymatic action. Pancreatic lipase, the main fat-digesting enzyme, cleaves triglycerides into monoglycerides and fatty acids. These smaller molecules are then further broken down by intestinal lipases into free fatty acids.

### ***Absorption of Fats***

Free fatty acids and monoglycerides are incorporated into micelles, small spherical structures formed by bile acids, phospholipids, and cholesterol. Micelles solubilize the hydrophobic fatty acids in the aqueous environment of the small intestine, allowing them to be absorbed into enterocytes, specialized epithelial cells lining the small intestine.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

### ***Transport of Fats***

Within enterocytes, free fatty acids are re-esterified with monoglycerides to form triglycerides. These triglycerides are then packaged into lipoproteins, particles that transport lipids in the bloodstream. Two major types of lipoproteins are involved in fat transport: chylomicrons and verylow-density lipoproteins (VLDLs). Chylomicrons are synthesized in enterocytes and carry triglycerides absorbed from the diet to various tissues, including adipose tissue and muscle. VLDLs are produced in the liver and transport triglycerides synthesized by the liver to other tissues.

### ***Oxidation of Fats***

Fatty acids derived from dietary fats and triglycerides stored in adipose tissue can be broken down into acetyl-CoA, a key intermediate in energy production, through a process called  $\beta$ -oxidation.  $\beta$ -oxidation occurs in the mitochondria of cells and releases energy in the form of ATP, the cell's energy currency.

### ***Synthesis of Fats***

Excess carbohydrates and amino acids can be converted into fatty acids through a process called de novo lipogenesis, which primarily occurs in the liver and adipose tissue. De novo lipogenesis is an energy-intensive process that requires acetyl-CoA as a starting material.

### ***Regulation of Fat Metabolism***

The metabolism of fats is tightly regulated by a complex network of hormonal and other signaling molecules, including:

- Insulin: Insulin promotes glucose uptake into cells and inhibits fat breakdown.
- Glucagon: Glucagon stimulates fat breakdown to release fatty acids for energy production.
- Epinephrine: Epinephrine, also known as adrenaline, increases fat breakdown to provide energy during stress or exercise.
- Adiponectin: Adiponectin, an adipokine produced by adipose tissue, enhances insulin sensitivity and promotes fat metabolism.

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

- Peroxisome proliferator-activated receptors (PPARs): PPARs are transcription factors that regulate gene expression involved in fat metabolism. Understanding the biochemistry of fat metabolism in monogastric animals is crucial for maintaining their energy balance, preventing obesity, and promoting overall health. By understanding the factors that regulate fat metabolism, we can develop dietary and lifestyle strategies to optimize fat utilization and reduce the risk of metabolic disorders.

### ***Factors that Affect Fat Metabolism***

A number of factors can affect the metabolism of fat, including diet, exercise, and genetics. A diet high in fat can lead to an increase in fat storage, while a diet low in fat can lead to a decrease in fat storage. Exercise can also help to promote fat loss. Genetics can also play a role in fat metabolism, with some people being more predisposed to storing fat than others.

The metabolism of fats in monogastric animals involves a series of complex processes that allow them to efficiently utilize dietary fats for energy production and storage. It encompasses the digestion, absorption, transport, oxidation, and synthesis of fatty acids, the major components of fats.

## **6. WATER IN ANIMAL NUTRITION**

Water is essential for sustaining life and ranks second to oxygen in importance. Water is needed in greater quantity than any other orally ingested substance and is classified as a macronutrient. Sources of water include drinking water, metabolic water (produced during catabolism of carbohydrates, fats, and proteins to carbon dioxide and water), and the water that presents as moisture in different feed ingredients. Metabolic water serves as the sole source of water in desert and hibernating animals, and feed water is the major water source for marine animals.

## **7. WHY IS WATER IMPORTANT FOR LIVESTOCK?**

Water is important for all organisms. Water makes up one-half to two-thirds of the body mass of adult animals and more than 90% of the body mass of newborn animals.

## **CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION**

Water is an essential constituent of almost all secretions of the body. Within the body, water is a universal solvent that facilitates cellular biochemical reactions involving digestion, absorption, and transportation of nutrients. The aqueous medium of water helps different digestive juices and food components interact, enhancing digestion, and helps in the excretion of waste products in the form of urine, feces, and perspiration, sweat, from the animal body. Because of the high specific heat of water, it helps in regulating body temperature, by absorbing the heat generated through different metabolic reactions. Water also regulates body temperature through evaporation as sweat or transports heat away from organs through blood. Water provides shape to body cells. Water helps in maintaining the acid-base balance of the body. Water acts as a cushion for tissue cells and the nervous system and protects the various vital organs against shocks and injuries.

### **8. WATER SOURCES AND LOSSES**

The animal body derives water from different sources. These include drinking water, water present as part of feeds (moisture), or those liberated during several metabolic reactions. The importance of these different sources varies among species, habitat, and diet. For example, hibernating animals and desert rodents depend on metabolic water to keep them alive, whereas marine animals depend on their diet to derive their water requirements. Metabolic water also depends on the type of nutrient catabolized. Oxidation of fat produces the greatest amount of metabolic water. However, overall contribution of metabolic water to daily water needs is less than 5% to 10% in most animals. The water content of feed consumed by ruminant and nonruminant animals vary highly. Forages consumed by ruminant animals vary from 5% to 7% for mature plant products, such as hay, to more than 90% for young lush green vegetation. Animals such as sheep depend a lot on water derived from such green forages for their need. Most commercial diets fed to nonruminant animals such as pigs and poultry may contain 7% to 10% moisture. Some of the canned foods fed to pet animals such as dogs and cats may contain more than 75% moisture. All animals experience daily water loss through different venues such as urine, feces, sweat, saliva, evaporation from the lungs through respiration, and milk in lactating animals.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

Among these, urinary loss accounts for the major loss. Water lost through urine serves as a tool to dispose of the toxic products of metabolism. Some animals such as birds are capable of concentrating urine and excreting it as uric acid instead of urea and thus conserving water. Urinary water loss depends on weather and on the type of food consumed. Consumption of excess water during heat stress can increase urinary volume. Animals that consume high-fibrous diets excrete more water in their feces. Fecal water excretion is higher in cows (30%–32%) compared to sheep (13%–24%) that void pellet-type dry feces to minimize water loss. Sweating is a means to dissipate body heat. In animals such as horses, loss of water through sweating is high. Animals such as chickens and dogs have very poorly developed sweat glands and compensate for heat loss by panting and increasing water intake. Daily clean water consumption is needed to make up for all the losses and is extremely important during periods of heat stress, especially in animals such as poultry.

**Table 1.** Daily Water Requirements of Different Livestock Species

Animal	L/day	Gal/day
Beef cattle	26 - 66	6.5 - 17
Dairy cattle	38 - 110	9.5 - 28
Horses	30 - 45	7 - 12
Sheep and goats	4 - 15	1 - 4

### 9. WATER REQUIREMENTS

An animal's water requirement depends on several factors such as ambient temperature, diet (energy level, fiber content, salt), physiological state (age, growth, pregnancy, ability to conserve water), level of exercise, and health. Environmental temperature (and associated humidity) is a major factor contributing to water intake. Water consumption, when expressed by unit of body weight for non-heat-stressed, nonlactating cattle, is around 5% to 6% of the body weight per day (or 2–5 kg of water for every kg of dry feed consumed) and can go up to 12% or more under heat stress. Water intake increases with higher environmental temperatures and increasing physical activity because of water lost through evaporative loss.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

Dietary dry matter intake and feed water content are highly correlated with water intake at moderate temperatures. High-energy, high-fat, and high-protein diets increase water intake because of increases in metabolic waste and urinary excretion of urea as well as increases in heat produced by metabolism. The salt content of a diet increases water consumption. Diets high in fiber (bran, dry forages) increase water intake as well. Young animals have higher water requirements per body size as compared to large animals. Similarly, animals that conserve water, such as sheep and poultry, need lower levels than cattle. Pregnancy and milk production increases water intake too. Dairy cattle may require 38–110 L/d compared to beef cattle at 22–66 L/d.

### **10. WATER RESTRICTION AND TOXICITY**

Water shortage affects both domestic and wild animals. To compensate for the losses and to maintain all related physiological functions, animals should have access to a clean supply of water. Lack of enough water could lead to a reduction in feed intake and productivity. Dehydration of the body leads to a reduction in body weight and the consequences are worse in high environmental temperatures. Dehydration is accompanied by a loss of electrolytes, an increase in body temperature, and an increase in respiratory rate. Animals become highly irritable, and prostration and death follow after severe water deprivation. Water intoxication may occur as a result of a sudden ingestion of large amounts of water after a short period of deprivation and is due to the slow adaptation of the kidneys to the high water load. Water restriction reduces feed consumption and is very stressful for animals.

### **11. WATER QUALITY**

Water per se is almost nontoxic, but problems with water arise from contamination with microbes, parasites, minerals, and various other toxic substances, such as pesticides. Water quality affects consumption, productivity, and animal health. Substances such as salts, pathogenic organisms, algae, and pesticides pollute water supplies and can affect palatability.

Ambient temperature is the major factor affecting an animal's water intake. Other factors include age, type of diet, level of exercise, stage of growth, or pregnancy.

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

Mineral salts include carbonate and bicarbonates, sulfates, and chlorides of Ca, Mg, Na, and K. Other toxic substances in water include nitrate, iron salts, and hydrocarbons. Contamination with nitrate is common in farming intensive areas. Concentrations above 1,500 ppm may cause toxicity causing death from anoxia. Iron salts in groundwater cause rust deposits on pipes and may cause bacterial contamination by iron-utilizing bacteria. Pesticides such as malathion and organophosphates may get into water systems and can be toxic. Certain blue-green algae (cyanogenic) in lakes can produce toxic substances. Toxicity in livestock causing vomiting, frothing, muscle tremors, liver damage, and death are reported due to blue-green algae toxicosis.

Water quality can influence the development of polioencephalomalacia, a noninfectious disease affecting the brain in feedlot cattle. Most affected animals show aimless wandering, disorientation, blindness, recumbency, star-gazing posture, and edema in the brain, causing a “softness” in the brain. Water high in sulfates promotes polioencephalomalacia, apparently via a complex interaction with other minerals and B vitamins. Most domestic animals can tolerate a total dissolved solid concentration of 15,000 to 17,000 mg/L. Water containing less than 1,000 mg/L of total soluble salt is safe for all classes of livestock. At higher ( $> 5,000\text{--}7,000$  mg/L) levels, it may cause mild diarrhea and an increase in mortality in poultry, but it could be acceptable to other livestock. A guideline for the interpretation of total dissolved solids in water is shown in table 2.

Prolonged exposure to poor-quality water may also reduce feed intake and overall animal performance. Young, pregnant, or lactating animals are generally more sensitive to elevated mineral concentrations in drinking water. Regular monitoring of water quality is therefore essential in livestock production systems. Management strategies such as water treatment or alternative water sources can help minimize health risks. Ensuring adequate water quality contributes significantly to animal welfare and productivity.

## CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION

**Table 2.** Guidelines for interpretation of total dissolved solids content in water

Total dissolved solids, mg/L	Interpretation
<1000	Suitable for all classes of livestock
1000-1999	Satisfactory for all classes of livestock; may produce transient diarrhea in animals
2000-4999	Temporary water refusal and diarrhea may be seen when animals are exposed to such water sources. May reduce productivity in dairy cattle.
5000-6999	Likely to reduce productivity in dairy cattle. May reduce growth rates. May result in water refusal and diarrhea. Avoid if possible.
7000-10000	Unfit for swine, very risky in all other species. Avoid.
> 10000	Dangerous, avoid.

## CONCLUSION

Monogastric nutrition focuses on efficient digestion and absorption of carbs, proteins, and fats in the simple stomach and small intestine, alongside adequate water intake. Balancing essential amino acids, optimizing energy sources, and ensuring clean water support overall health, growth, and productivity in animals like pigs, poultry, and humans. Understanding these processes enables optimized diets, reduced waste, and improved animal performance.

### ***Key Points***

Water is one of the most important nutrients, yet it is almost always neglected. Water serves as the fluid matrix of the animal body. Water gives form and structure and provides protection from environmental stress. The high solvent power of water permits the formation of solutions within which metabolic reactions occur.

- There are different sources of water (e.g., diet, drinking, metabolic).
- Metabolic water is produced during catabolism of carbohydrates, fats, and proteins to carbon dioxide and water and is important for hibernating animals. Species difference, type of metabolism, and digestive tract type affect water requirements. For example, birds and fish have low

## ***CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL NUTRITION***

requirements, while ruminants need a large quantity of water to suspend ingesta in the rumen.

- An animal's water requirement is highly influenced by environmental temperature, humidity, diet (energy level, fiber content, salt), physiological state (age, growth, pregnancy), and level of exercise and health.
- Water restriction affects animal health, growth, and productivity and is very stressful for animals.
- Water containing less than 1,000 mg/L of total soluble salt is safe for all classes of livestock.

### ***Review Questions***

Water is one of the most important essential nutrients. Functions of water include:

- Can act as a diluent
- Carrier of waste from the body
- Transport of nutrients
- All of the above
- List the sources of water in the animal body.
- Major loss of water in a beef cattle is through which medium?
- Water quality can affect cattle health. Name a non infectious disease in feedlot cattle associated with water quality.

**CLIMATE CHANGE IMPACTS ON AGRICULTURE AND ANIMAL  
NUTRITION**

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