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*Research article*

# THE EFFECT OF DIFFERENT FORMS OF SOLID FEED ON BIOCHEMICAL PARAMETERS IN BLOOD PLASMA OF CALVES

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The study examined how different solid feeds affected the biochemical parameters in calf plasma. The experiment involved a control group and three test groups of calves, each fed with a different starter mixture. The results showed significant differences in some biochemical parameters between the feeding groups. Calves in groups P1 and P3 had a statistically higher glucose concentration in blood plasma compared to group C. Calves from group P3 had a higher concentration of urea in blood plasma than calves from group C. Calves in groups P1 and P3 also had higher concentrations of total protein and globulin in blood plasma compared to group C. The concentration of inorganic phosphate in the blood plasma of calves from group P3 was significantly higher than that of group C. Female calves in the experimental groups showed a lower concentration of NEFA at three months of age compared to the control group. It was concluded that feeding calves with a starter mixture containing whey and easily digestible protein had a positive effect on the nutritional status and energy balance of the calves.

**Keywords:** biochemical parameters, blood plasma, calf, feeding regimes

## INTRODUCTION

The analysis of plasma biochemistry is a key aspect of a mammal's overall health and physiological state. Various biochemical parameters, including glucose, total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, triglycerides, electrolytes such as sodium, potassium, and chloride, calcium, phosphate, and creatine kinase (CK), can be measured in the plasma. Glucose is an essential source of energy for the body and serves as a parameter of blood sugar levels. Total protein is a combination of albumin and globulin and provides valuable insights into the nutritional and immune status of the mammal. Albumin is a specific protein that plays an instrumental role in maintaining osmotic pressure and

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transporting various substances in the blood. Globulins include antibodies and other proteins that are involved in immune responses. BUN is an important parameter of kidney function and protein metabolism, while creatinine is another marker for kidney function. Total bilirubin, a by-product of red blood cell breakdown, can be a parameter of liver or gallbladder issues if present in elevated levels. ALT is an enzyme associated with liver function, and AST is another enzyme that indicates liver and muscle health. ALP is an enzyme associated with bone and liver activity, while cholesterol is vital for cell membrane structure and hormone production. Triglycerides are the storage form of fat and an energy source. Electrolytes such as sodium, potassium, and chloride are essential for maintaining fluid balance and nerve function. Calcium and phosphate are essential for bone formation and other physiological processes, and CK is an enzyme that can indicate muscle damage. Normal biochemical values in plasma or blood serum are important to confirm various disease states in the body, with the fact that the values in calves are not the same as in adult cattle [1,2]. The changes that occur in the first days of life are due to adaptation to extrauterine life and largely depend on the growth and development of organs and the intake of nutrients. Veterinarians often use blood tests to measure these parameters and gain an insight into the calf's overall health, nutritional status, and potential presence of diseases or abnormalities. It is essential to interpret the results in the context of the specific animal being evaluated, considering factors such as age, breed, and sex [3]. Regular monitoring of these biochemical parameters is critical for early detection of any health issues and effective management of calf health. By keeping track of these parameters, veterinarians can provide the necessary care to maintain the calf's health and well-being.

Gamma-glutamyl transferase (GGT) is an enzyme that plays an important role in the body [4]. It is primarily produced in the bile duct and kidneys, and an increased level of GGT in the bloodstream can indicate damage to the bile duct. Additionally, GGT levels are often high in young calves that have been suckled on colostrum, which is a nutrient-rich substance produced by cows after giving birth. This is because bovine colostrum contains a large amount of GGT that passes through the intestinal wall and enters the bloodstream of the calf. As a result, the concentration of GGT in the blood can be used as a helpful indicator of whether the calf received enough colostrum [5,6]. GGT levels in the bloodstream tend to decrease rapidly after birth and stabilize after 20 days. Therefore, it's important to measure GGT levels early in the calf life. In general, GGT values at the beginning of a calf's life should not be below 200 units. If the GGT level is too low, this could be a sign that the calf did not receive enough colostrum [6,7]. By monitoring GGT levels in young calves, farmers and veterinarians can ensure that calves are getting the nutrition they need to grow and thrive.

Insulin-like Growth Factor-1 (IGF-1) is a vital growth factor that plays a crucial role in regulating the growth of animals during the neonatal period. It is a component of the hypothalamic-pituitary axis, which, in conjunction with various hormones and growth factors, contributes to the proper development and growth of the animal's body [8-10]. Veterinary practitioners can measure IGF-1 levels in the blood to evaluate

the growth and nutritional status of animals, particularly calves. Low levels of IGF-1 may indicate issues with the production or responsiveness of the growth hormone, malnutrition, or other health concerns. It is important to note that various factors such as age, sex, and nutritional status can influence IGF-1 levels. Monitoring IGF-1 levels can be an essential tool for managing the health and growth of calves, especially in the context of livestock farming or veterinary care. The analysis of 12 fattened calves with an average age of 45 days and weighing 54.6 kg over 336 days indicated that there is a strong correlation between the concentration of IGF-1 in the plasma and the average daily gain and that plasma IGF-1 levels can be used to determine the fattening characteristics of animals [11].

Furthermore, calves require proper nutrition to grow and develop optimally. Feeding plays a critical role in their growth rate, overall health, and immune system strength. Therefore, it is essential to monitor various biochemical parameters during calf feeding to ensure their health and proper growth [12]. The research study conducted by Moeini et al. [13] examined the impact of various physical forms of food on biochemical parameters in the blood of calves. The study compared the effects of ground, textured, pelletized, and ground starter mixture with the addition of alfalfa hay. The results of the study indicate that among the groups, the calves fed with ground starter mixture with the addition of alfalfa hay had the highest values of glucose concentration ($P < 0.01$) and triglycerides ($P < 0.05$) in the blood. Conversely, the group that was fed with pelleted starter mixture had significantly lower values of glucose concentration and triglycerides ($P < 0.01$; $P < 0.05$). Many studies have confirmed that adequate calf growth is crucial for successive milk production. Chester-Jones et al. [14] conducted a study to determine the effect of growth, feed consumption, and calving season on the performance of Holstein cows during their first lactation. They monitored 2,880 calves to assess their daily gain, body mass, consumption of milk substitute and starter mixture, and calving season. The calves were weaned at six weeks of age, and their average weight was found to be 62.4 kg. The daily gain was 0.53 kg/day, and by the sixth week of life, they had consumed 21.5 kg of milk substitute and 17.3 kg of starter mixture. They concluded that the consumption of the starter mixture, the body weight of the calves, and the average daily gain at six and eight weeks had a significant impact on the amount of milk, milk fat, and protein produced during the first lactation. Calves born in spring and summer had lower starter dry matter consumption, body weight, and average daily gain at eight weeks of age. Furthermore, achieving average daily gains of more than 0.5 kg/day until weaning could significantly affect the performance of Holstein cows during their first lactation [15]. Rauba et al. [16] collected data on the consumption of protein and metabolic energy from milk replacer and starter mixture for 4,534 Holstein heifers. They calculated the amount of metabolic energy and protein consumed by each calf at six and eight weeks of age. Also, they found that the consumption of protein and metabolic energy from the starter mixture had a greater impact on calf growth than the consumption of protein and metabolic energy from the milk substitute. This is because the amount of milk

substitute was fixed for most calves. They also determined that greater consumption of the starter mixture had a positive impact on milk production in the first lactation, including the amount of milk, fat, and protein.

Considering the importance of adequate feeding and growth of calves on the potential and realization of future milk production, this research aimed to compare different mixtures and determine the optimal ones based on the values of biochemical parameters measured during the initial phase of calf growth.

## MATERIAL AND METHODS

### Experiment plan

The study aimed to investigate the feeding patterns of calves and their growth rate during a three-month period from June 2019 to September 2019. The study included a total of 40 calves divided into four groups of ten based on their feeding regimen. The feeding regimen consisted of a milk substitute, which was a high-quality protein source derived from whey protein with a digestibility rate of approximately 98% and hydrolyzed wheat protein with a digestibility rate of about 92%. Additionally, the milk substitute fat contained 40% coconut oil and 60% palm oil, which was the ideal ratio for achieving the desired ratio of fatty acids in the milk. Furthermore, the fat was homogenized to form small balls with a diameter of less than 3 μm, which made it easily digestible, with a digestibility rate of about 87%.

Starting from the fourth day of life, solid food, in the form of pelleted starter mixture, was offered to the calves ad libitum. Water was also available to the calves ad libitum from the fourth day of age. The starter mixture was pelletized on a Bühler pelletizer, using a 4.5 x 60/50 matrix, achieving a Pellet Durability Index (PDI) of 95 for all four mixtures used in the study. The quality of the pelletized starter mixture was found to be highly critical since calves consume more feed in pelleted form than in mealy form, and with pelleted fodder mixture, there is less waste of feed by the animal. All four produced starter mixtures had uniform nutritional value, but different raw material composition. Various additives were used in the raw material composition of the mixture, depending on the feeding group of the animals (mannan-oligosaccharides, nucleotides, and soy protein concentrate).

The starter mixtures of all four nutritional groups contained a commercial yeast-based product produced from the yeast wall of *Sacharomyces cerevisae*, which is rich in ***mannan-oligosaccharides*** (MOS). MOS helps defend the gastrointestinal tract against pathogenic microorganisms. ***Nucleotides***, another commercial yeast-based product produced from an extract of the yeast Saccharomyces cerevisiae, were used in starter mixtures P2 and P3 of the nutritional group. The product contains a significant proportion of nucleic acids in its composition (over 5%) and is rich in proteins. ***Soy protein concentrate***, a commercial product with a protein digestibility rate of about

96%, was used in starter mixtures P2 and P3 of the nutritional group. Compared to soybean meal, soy protein concentrate has a significantly lower proportion of anti-nutritional factors (ANF), such as trypsin inhibitors, glycinin, and β-conglycinin.

The experiment involved the control and three test groups of calves, each fed with a different starter mixture (Table 1). Control group (C) was fed with standard feed mixture for calves. Group P1 was given a starter mixture containing dry sweet whey as a source of lactose, replacing a part of the corn. Group P2 was fed a starter mixture in which soybean meal and rapeseed meal were replaced with other protein sources, including yeast nucleotides, soy protein concentrate, methionine, and lysine. Group P3 was given a starter mixture containing dry whey, yeast nucleotides, soy protein concentrate, methionine, and lysine.

**Table 1.** Experimental scheme

| Group | No animals | Feed mixture |
|---|---|---|
| **C, control** | 10 | Milk substitute + GT-1 18% CP, |
| **P1, experimental 1** | 10 | Milk substitute + GT-1 18% CP, whey |
| **P2, experimental 2** | 10 | Milk substitute + GT-1 18% CP, nucleotides, soy protein concentrate, and amino acids |
| **P3, experimental 3** | 10 | Milk substitute + GT-1 18% CP, whey, nucleotides, soy protein concentrate, and amino acids |

*GT-1 – standard feed mixture for calves; CP – crude protein

During the study, all feeding groups of calves were weaned at an average age of 56 days. The consumption of the milk substitute was closely monitored on a daily basis. For each calf, the amount of milk substitute offered to them was recorded, and the difference between the amount of food offered and the rest of the food was calculated until weaning. Similarly, the consumption of the starter mixture was also monitored. Each calf was offered a weighed amount of the mixture every day, and the next day at the same time, the remains were weighed. This enabled to calculate the difference between the amount of the mixture offered and the remains of the mixture. After weaning, all calves were fed a total mixed ratio (TMR), which provided them the appropriate nutrients for their growth and development (Table 2).

**Table 2.** The content of total mixed ratio (TMR)

| Feed | kg / FD (feeding day) | Chemical composition | % |
|---|---|---|---|
| **Hay** | 0.5 | Dry matter, DM | 66.2 |
| **Corn silage** | 2.3 | Crude protein, CP (%) | 16.3 |
| **High moisture corn** | 0.9 | NDF (%) | 24.6 |
| **GT-2 21% CP** | 2.5 | Crude fat (%) | 3.1 |
| **Total** | 6.2 | Starch (%) | 38.8 |
| | | Crude ash, CA (%) | 5.7 |
| **Metabolic energy, ME** | 12.4 MJ/kg DM | Ca (%) | 0.7 |
| | | P (%) | 0.5 |

***GT-2** – standard feed mixture for weaned calves

Furthermore, blood samples were collected from the calves at four different times. The first blood sample was taken when the calves were about 6 days old on average. The second sample was collected when the calves were about 24 days old on average. The third sample was collected when the calves were about 50 days old on average. The fourth and final sampling was done only on female calves when they reached an average age of 91 days. Blood was taken from the jugular vein, and the Vacutainer® system was used. After blood collection, the samples were centrifuged using a 32 Rotofix A device (Andreas Hettich GmbH&Co, Germany) at 1,500 revolutions for 10 minutes. The blood plasma was then separated using a pipette (Hirschmann Laborgeräte GmbH & Co. KG) with a disposable tip, with 1.5 ml of plasma stored in a microtube (Eppendorf AG, Germany) and frozen at – 80°C until analysis. Biochemical analyses were performed on a biochemical analyser (Beckman Coulter AU400, USA) and following biochemical parameters were determined: gamma-glutamyl transferase (GGT), glucose, urea, total proteins, albumins, globulins (calculated as the difference between the concentration of total proteins and albumin), β-hydroxybutyric acid BHB), non-esterified fatty acids (NEFA), insulin-like growth factor 1 (IGF-1), mineral concentrations (iron, calcium) and inorganic phosphate.

## Statistical analysis

For the logical control of the dataset and statistical analysis of the determined biochemical parameters in blood plasma, SAS/STAT [17] was used. For the evaluation of the significance of the effect of different feeding treatments (C, P1, P2, and P3) on the variability of blood biochemical parameters (gamma-glutamyl transferase, glucose, urea, total proteins, albumins, globulins, β-hydroxybutyric acid, non-esterified

fatty acids, insulin-like growth factor 1, mineral concentrations (iron, calcium) and inorganic phosphate), the following statistical model was used:

$$y_{ijklm} = \mu + b_1 d_i + b_2 d_i^2 + T_j + S_k + M_l + e_{ijklm}$$

Where:

$\mathbf{y_{ijklm}}$ = estimated biochemical parameter;

$\mu$ = intercept;

$\mathbf{b_1}$, $\mathbf{b_2}$ = regression coefficient;

$\mathbf{d_i}$ = calf age (i = 1 – 96 days);

$\mathbf{T_j}$ = fixed effect of treatment j (j = C, P1, P2, P3);

$\mathbf{S_k}$ = fixed effect of calf gender k (k = male, female);

$\mathbf{M_l}$ = fixed effect of number of sampling l (l = 1, 2, 3, 4);

$\mathbf{e_{ijklm}}$ = error.

The significance of the differences in the estimated LsMeans of the analysed parameters due to the effect of the treatment was tested by Scheffe's multiple comparison method using the PROC GLM procedure in SAS [17].

Furthermore, the significance of the effect of different feeding treatments (C, P1, P2, and P3) on the variability of biochemical parameters in plasma was also evaluated separately by the number of blood sampling (1., 2., 3., 4.) using the following statistical model:

$$y_{ijkl} = \mu + b_1 d_i + b_2 d_i^2 + T_j + S_k + e_{ijkl}$$

Where:

$\mathbf{y_{ijklm}}$ = estimated biochemical parameter;

$\mu$ = intercept;

$\mathbf{b_1}$, $\mathbf{b_2}$ = regression coefficient;

$\mathbf{d_i}$ = calf age (i = 1 – 96 days);

$\mathbf{T_j}$ = fixed effect of treatment j (j = C, P1, P2, P3);

$\mathbf{S_k}$ = fixed effect of calf gender k (k = male, female);

$\mathbf{e_{ijklm}}$ = error.

The significance of the differences in the estimated values of the analysed parameters due to the effect of treatment separately by ordinal number of blood sampling was tested by Scheffe's multiple comparison method using the PROC GLM procedure in SAS (SAS Institute Inc., 2019).

## RESULTS

Estimated LsMeans of biochemical parameters in calf plasma (gamma-glutamyl transferase, glucose, urea, total protein, albumins, globulins, iron, inorganic phosphate, calcium, non-esterified fatty acids, beta-hydroxybutyric acid and insulin-like growth factor 1) regarding the nutritional group are presented in Table 3. Estimated LsMeans of urea and NEFA differed statistically significantly ($P < 0.05$) regarding the feeding group. The highest estimated LsMeans of urea were measured in groups P3 (3.36 mmol/L) and P2 (3.32 mmol/L) and were statistically significantly higher ($P < 0.05$) than urea values in the C group (2.35 mmol/L). The urea value in the P1 group (3.21 mmol/L) was not statistically significantly ($p > 0.05$) different from the values in the other groups. The highest value of NEFA was measured in the C group (0.21 mmol/L) and was statistically significantly higher ($P < 0.05$) than the average value of NEFA measured in groups P2 (0.16 mmol/L), P3 (0, 13 mmol/L) and P1 group (0.12 mmol/L). Furthermore, other analyzed biochemical parameters of calf plasma (GGT, glucose, total protein, albumins, globulins, iron, inorganic phosphate, calcium, BHBA and IGF-1) did not differ statistically significantly ($P < 0.05$) depending on the feeding group.

**Table 3.** LsMeans of biochemical parameters in calf plasma regarding the feeding group (n = 40)

| Parameter | C | P1 | P2 | P3 |
|---|---|---|---|---|
| **Gamma-glutamyl transferase (GGT), U/L** | 102.18 | 102.06 | 110.83 | 64.82 |
| **Glucose, mmol/L** | 6.05 | 6.85 | 6.86 | 6.28 |
| **Urea, mmol/L** | $2.35^{A}$ | $3.21^{AB}$ | $3.32^{B}$ | $3.36^{B}$ |
| **Total protein, g/L** | 67.90 | 72.30 | 71.25 | 68.60 |
| **Albumin, g/L** | 32.63 | 35.02 | 34.34 | 34.70 |
| **Globulin, g/L** | 35.27 | 37.28 | 36.92 | 33.89 |
| **Iron, Fe, μmol/L** | 40.41 | 34.01 | 35.23 | 33.19 |
| **Inorganic phosphorus, P, mmol/L** | 2.86 | 3.07 | 3.15 | 3.17 |
| **Ca, mmol/L** | 2.94 | 3.08 | 3.12 | 3.02 |
| **Non-esterified fatty acids (NEFA), mmol/L** | $0.205^{A}$ | $0.117^{B}$ | $0.151^{B}$ | $0.127^{B}$ |
| **Beta-hydroxybutyric acid (BHBA), mmol/L** | 0.112 | 0.122 | 0.119 | 0.125 |
| **Insulin-like growth factor 1 (IGF-1), ng/mL** | 40.27 | 44.75 | 36.33 | 46.23 |

*values in the same row marked with different letters differ statistically significantly ($P < 0.05$)

The estimated LsMeans of biochemical parameters in calf plasma regarding the number of blood sampling/age (first/age of 6 days, second/age of 24 days and third/age of 50 days blood sampling for both male and female calves (n = 40), and the fourth/age of age 91 days blood sampling only for female calves (n = 20)), and the feeding group are presented in Table 4.

**Table 4.** LsMeans of biochemical parameters in calf plasma regarding the sampling number (calf age) and the feeding group

| Age of calf | Feeding group | | | | Feeding group | | | |
|---|---|---|---|---|---|---|---|---|
| | C | P1 | P2 | P3 | C | P1 | P2 | P3 |
| **Gamma-glutamyl transferase (GGT), U/L** | | | | | Iron, Fe, µmol/L | | | |
| **1. (6.day)** | 280.10 | 324.17 | 295.05 | 235.43 | 34.67 | 22.61 | 25.58 | 25.14 |
| **2. (24.day)** | 61.48 | 64.09 | 59.11 | 45.19 | 50.84 | 43.28 | 38.59 | 43.65 |
| **3. (50.day)** | 19.51 | 26.50 | 25.73 | 27.56 | 30.96 | 31.12 | 24.21 | 29.06 |
| **4. (91.day)** | 24.43 | 29.92 | 19.30 | 26.72 | 38.49 | 31.89 | 32.65 | 25.49 |
| **Glucose, mmol/L** | | | | | Inorganic phosphorus, P, mmol/L | | | |
| **1. (6.day)** | 5.23 | 5.84 | 6.63 | 6.19 | 2.56 | 2.60 | 2.79 | 2.91 |
| **2. (24.day)** | 6.64 | 7.58 | 6.97 | 5.73 | 3.05 | 3.19 | 3.16 | 2.92 |
| **3. (50.day)** | $4.51^{A}$ | $6.17^{B}$ | $5.12^{AB}$ | $6.04^{B}$ | $2.67^{A}$ | $3.04^{AB}$ | $2.79^{AB}$ | $3.64^{B}$ |
| **4. (91.day)** | 6.06 | 6.06 | 6.17 | 5.58 | 2.88 | 3.22 | 3.04 | 2.68 |
| **Urea, mmol/L** | | | | | Ca, mmol/L | | | |
| **1. (6.day)** | 2.24 | 3.74 | 3.10 | 3.51 | 2.78 | 2.76 | 2.98 | 2.92 |
| **2. (24.day)** | 2.35 | 2.21 | 2.17 | 2.15 | 2.94 | 3.02 | 2.94 | 2.80 |
| **3. (50.day)** | $3.35^{A}$ | $4.19^{AB}$ | $5.36^{AB}$ | $6.10^{B}$ | 2.58A | $3.14^{B}$ | $2.77^{AB}$ | $3.15^{B}$ |
| **4. (91 day)** | 3.15 | 5.10 | 4.97 | 3.00 | 3.11 | 2.91 | 3.00 | 2.62 |
| **Total protein, g/L** | | | | | Non-esterified fatty acids (NEFA), mmol/L | | | |
| **1. (6.day)** | 64.69 | 63.91 | 67.73 | 60.47 | 0.273 | 0.149 | 0.192 | 0.133 |
| **2. (24.day)** | 68.32 | 68.71 | 67.06 | 62.08 | $0.159^{A}$ | $0.059^{B}$ | $0.120^{AC}$ | $0.096^{BC}$ |
| **3. (50 day)** | $59.60^{A}$ | $76.37^{B}$ | $70.23^{AB}$ | $81.18^{B}$ | 0.124 | 0.073 | 0.118 | 0.079 |
| **4. (91.day)** | 83.12 | 83.67 | 76.30 | 68.73 | $0.194^{A}$ | $0.084^{B}$ | $0.102^{B}$ | $0.093^{B}$ |
| **Albumin, g/L** | | | | | Beta-hydroxybutyric acid (BHBA), mmol/L | | | |
| **1. (6. day)** | 27.32 | 28.28 | 29.29 | 29.93 | $0.033^{A}$ | $0.046^{AB}$ | $0.044^{AB}$ | $0.053^{B}$ |
| **2. (24.day)** | 32.06 | 33.18 | 32.67 | 32.12 | 0.047 | 0.037 | 0.049 | 0.030 |
| **3. (50.day)** | $30.97^{A}$ | $37.20^{AB}$ | $32.71^{AB}$ | $38.24^{B}$ | 0.122 | 0.128 | 0.093 | 0.133 |
| **4. (91.day)** | 36.00 | 36.02 | 34.98 | 31.45 | 0.204 | 0.249 | 0.247 | 0.249 |
| **Globulin, g/L** | | | | | Insulin-like growth factor 1 (IGF-1), ng/mL | | | |
| **1. (6.day)** | 37.28 | 35.63 | 38.43 | 30.53 | 42.81 | 47.65 | 32.99 | 62.94 |
| **2. (24.day)** | 36.25 | 35.53 | 34.39 | 29.96 | 44.45 | 50.01 | 25.43 | 26.98 |
| **3. (50 day)** | $28.63^{A}$ | $39.17^{B}$ | $37.53^{AB}$ | $42.94^{B}$ | 30.85 | 26.36 | 37.95 | 39.46 |
| **4. (91 day)** | 50.13 | 47.65 | 41.32 | 37.28 | 21.09 | 28.15 | 27.16 | 24.10 |

*values in the same row marked with different letters differ statistically significantly ($P < 0.05$)

The analysis showed that there was a statistically significant difference ($P < 0.05$) in the estimated mean values for BHBA for the different feeding groups at the first blood sampling, which occurred when the calves were six days old. Also, there was a statistically significant difference ($P < 0.05$) in the level of glucose, inorganic phosphate, urea, calcium, total protein, albumin and globulin in samples taken at 50 days. However, the other analysed biochemical parameters of calf plasma (GGT, glucose, urea, total protein, albumins, globulins, iron, inorganic phosphate, calcium, NEFA and IGF-1) taken at other calf age did not differ statistically significantly ($P > 0.05$) depending on the feeding group.

## DISCUSSION

The analysis showed that there was a statistically significant difference ($P < 0.05$) in the estimated LsMean values for BHBA for the different feeding groups at the first blood sampling, which occurred when the calves were six days old. However, the other analysed biochemical parameters of calf plasma (GGT, glucose, urea, total protein, albumins, globulins, iron, inorganic phosphate, calcium, NEFA and IGF-1) did not differ statistically significantly ($P> 0.05$) depending on the feeding group. Furthermore, analysed biochemical parameters were within normal physiological ranges, with the exception of the level of GGT, BHBA and IGF-1. Reported referent values for total proteins were higher than 60 g/L, for glucose 4.18 to 5.4 mmol/L, for albumin an average concentration of 24.7 g/L, for iron of 24.3 – 35.1 μmol/L, for inorganic phosphate of 2.0 – 2.91 mmol/L, for calcium in the interval 2.5 – 2.89 mmol/L [18]. The concentration of total protein in this research agreed with the concentrations determined in the research by Yu et al. [19], who measured a total protein concentration of 60.2 g/L in calves at 24 hours of age. The range of LsMean values for GGT was recorded from 235.43 U/L in the P3 group to 324.17 U/L in the P1 group. These findings were in line with the values presented in earlier studies [20], where the measured values for GGT in the first week of calf life were 329.8 ± 358.1 U/L. Moreover, it was found that such high values for GGT are normal for calves that have been fed a sufficient amount of colostrum because the colostrum of cows contains very high amounts of GGT, which was published by Hammon and Blum [21], where they determined an average value for GGT of 22.432 U/L colostrum. The highest concentration of BHBA was determined in the P3 group (0.053 mmol/L), which was statistically significant ($P < 0.05$) higher compared to the C group (0.033 mmol/L). P1 group (0.046 mmol/L) and P2 group (0.044 mmol/L) did not differ statistically significantly ($P < 0.05$) from the other two feeding groups. Higher BHBA concentrations indicate that calves adapt the organism to use another energy source, not glucose only. The concentration of NEFA in the plasma of calves was the highest at the first measurement, which agrees with the results published by Quigley et al. [22], where the plasma NEFA concentration in the first week was 0.256 mmol/L. Relatively high concentrations of IGF-1 in the plasma of six-day-old calves were

probably a reflection of good colostrum management since research [23] concluded that the concentration of IGF-1 in plasma is dependent on the consumption of colostrum. It was expected that all biochemical parameters would be similar and in range, for 6 days calves because they don't eat enough solid food and differences between concentrations of biochemical parameters can come mainly from differences in consumption of the mother's colostrum.

During the second blood sampling, which happened when the calves were 24 days old, the LsMeans for the NEFA concentration revealed statistically significant ($P < 0.05$) differences among the feeding groups. The NEFA concentration values indicated that the C group had the highest value (0.159 mmol/L), which was significantly higher ($P < 0.05$) when compared to the P1 (0.059 mmol/L) and P3 (0.096 mmol/L) groups. On the other hand, the P2 group (0.120 mmol/L) had a higher value ($P < 0.05$) when compared to the P1 group, but not when compared to the C and P3 groups. It is important to note that plasma NEFA is a direct indication of energy balance, and the concentration of NEFA increases as negative energy balance becomes more severe. Therefore, based on measurements of daily feed consumption, it could be observed that calves in the C group seemed to have eaten less pelleted starter, which led to a state of nutritional disbalance and lack of energy. However, the other analysed biochemical parameters of calf plasma, such as GGT, glucose, urea, total protein, albumins, globulins, iron, inorganic phosphate, calcium, BHBA, and IGF-1 did not differ significantly ($P > 0.05$) among the feeding groups and were within the physiological ranges for three-week-old calves. For instance, the measured values of GGT in the third week of calf life ($45.1 \pm 34.4$ U/L) agree with the results of study by Klinkon and Ježek [20]. Regarding the estimated mean values of urea in this research, they were below the lower limit of the reference values, while the total protein concentration values were slightly higher than the concentrations reported in research [19]. However, total protein concentration values were consistent with the reference interval [18]. It is worth mentioning that at 24 days of age, calves do not consume enough solid feed, and their nutritional requirements mainly come from a liquid diet. In this study, a solution of milk replacer was used to provide the necessary nutrients to the calves.

At the third blood sampling with 50 days of calf age, it was recorded that analysed biochemical parameters of calf plasma (GGT, iron, NEFA, BHBA and IGF-1) were similar in all feeding groups without statistical differences and they were within the normal physiological range. Furthermore, older calves eat larger amounts of solid feed, and because of that, statistically significant ($P < 0.05$) different values for glucose, urea, inorganic phosphate, calcium, total protein, albumin and globulin concentration regarding the feeding group were determined. The concentration of glucose in the plasma of calves was significantly ($P < 0.05$) higher in groups P1 and P3, which were fed with the starter which included dry whey, rich in lactose as the primary source of energy for calves. Urea was significantly ($P < 0.05$) higher in group P3 in comparison with the C group, and an increased value was found in group P2. Both groups, P2

and P3 were fed a starter mixture which included easily digested protein sources. Klinkon and Ježek [20] in their work published the mean average value for urea in six-week-old calves of 3.79 ± 1.26, which is within the reference values of 2.5 to 6.6 mmol/L [18]. Inorganic phosphate was significantly ($P < 0.05$) higher in group P3 in comparison with group C, and an increased value was also found in group P1 in comparison with group C. Both groups, P3 and P1 were fed a starter with included lactose, which provides a better environment for bacteria which produce the enzyme phytase [24], and in that way influences better bioavailability of phosphate. Similarly, calcium concentration was significantly higher ($P < 0.05$) in groups P1 and P3 in comparison with group C. The highest total protein concentration value was determined in the P1 and P3 groups in comparison with group C. Calves in groups P1 and P3 were fed a pelleted starter which has lactose in recipes, as an energy source for microbes in rumen. The concentration of total protein in all groups in this research was higher than those published in [19], where a total protein concentration of 48.0 g/L in seven-week-old calves, which was a lower value than the reference value [18]. Similarly, concentrations of albumin and globulin were significantly ($P < 0.05$) higher in group P3 in comparison with group C, while the concentration of globulin was significantly ($P < 0.05$) higher in P1 than in group C.

At the fourth blood sampling that occurred at 91 days of calf age, it was recorded that analysed biochemical parameters of calf plasma (GGT, glucose, urea, total protein, albumins, globulins, iron, inorganic phosphate, calcium, BHBA and IGF-1) were similar in all feeding groups without statistically significant ($P > 0.05$) differences. Also, analysed biochemical parameters were in normal physiological ranges for 3-month-old calves. Only NEFA was significantly higher ($P < 0.05$) in group C in comparison with all three experimental groups. Observed a post-weaning decrease in NEFA concentrations indicate that the transition to a solid-feed diet was successful [25]. Lower NEFA values indicate that calves in experimental groups have better nutritional status than calves in the control group. Higher NEFA concentration in plasma is related to energy mobilization as a consequence of acute glucose shortage in plasma. Furthermore, when glucose is in shortage, lipolysis generates NEFA which is transported to the liver where they are oxidized to energy or they become converted into ketone bodies [26].

## CONCLUSION

This study evaluated the impact of different pelleted starter mixtures on biochemical parameters in calf plasma, measured four times during the initial growth phase. Statistically significant differences were observed in glucose, urea, total proteins, albumins, globulins, inorganic phosphate, and calcium levels between feeding groups. Calves in groups P1 and P3 exhibited higher plasma glucose levels compared to group C at 50 days, suggesting improved energy supply due to the inclusion of dried whey. Group P3 calves also had significantly higher urea concentrations, indicating enhanced protein metabolism. While group P2 showed higher urea levels than groups C and

P1, this difference was not statistically significant. Higher total protein and globulin levels in groups P1 and P3 implied superior protein supply and utilization. Group P3 calves also had significantly higher inorganic phosphate levels, indicating better phosphate bioavailability likely due to phytase-producing bacteria. Female calves in the experimental groups had significantly lower NEFA concentrations at three months, reflecting better nutritional status. Overall, the inclusion of whey and easily digestible proteins in starter mixtures positively influenced the nutritional status and energy balance of calves.

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**Ethical Statement**

The study and collection of blood samples were conducted in strict compliance with the regulations and approval of the Bioethical Committee for Research on Animals at the Faculty of Agrobiotechnical Sciences Osijek. This ensured that all research activities were carried out ethically and with utmost care and responsibility towards the animals involved.

**Authors' contributions**

ZS participated in the experiment, designed and wrote the manuscript. SB participated in the experiment, helped to draft the manuscript. JN participated in the experiment. RG helped to draft the manuscript. MS participated in the analysis of the results. VG performed the statistical analysis, review and editing of the manuscript. All authors read and approved the final manuscript.

**Declaration of interest**

The contact author has declared that none of the authors has any competing interests.

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## UTICAJ RAZLIČITIH OBLIKA ČVRSTE HRANE NA BIOHEMIJSKE PARAMETRE U KRVNOJ PLAZMI TELADI

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Studija je se bavila ispitivanjem uticaja različitih čvrstih hraniva na biohemijske parametre u plazmi teladi. Eksperiment je uključivao kontrolnu grupu i tri test grupe teladi, od kojih je svaka hranjena različitom starter smešom. Rezultati su pokazali značajne razlike u nekim biohemijskim parametrima između grupa. Telad u grupama P1 i P3 imala su statistički veću koncentraciju glukoze u krvnoj plazmi u odnosu na grupu C. Telad iz grupe P3 imala su veću koncentraciju uree u krvnoj plazmi od teladi iz grupe C. Telad u grupama P1 i P3 takođe su imala veće koncentracije ukupnih proteina i globulina u krvnoj plazmi u poređenju sa grupom C. Koncentracija neorganskog fosfata u krvnoj plazmi teladi iz grupe P3 bila je značajno veća nego u grupi C. Ženska telad u oglednim grupama pokazala je nižu koncentraciju NEFA sa tri meseca starosti u poređenju sa kontrolnom grupom. Zaključeno je da ishrana teladi starter smešom koja

sadrži surutku i lako svarljive proteine pozitivno utiče na nutritivni status i energetski bilans teladi.