

**Research Article** 





# Blood parameters of male and female Holstein calves at birth and weaning in Mediterranean climate conditions

#### Abstract

Blood parameters are important for monitoring the health status, growth perfomance, nutritional status, and early of problem diagnosis. Blood parameters provide important information on the metabolic, immunological and nutritional status of calves. For this reason, there is a need to determine the ideal values of blood values obtained under different conditions in calves and to determine blood values for herd management planning. The study was carried out with 54 healthy Holstein calves blood sample between October and May in an intensive dairy herd in the Mediterranean region. The average birth weight of the calves was 37.52±0.424 kg and the average weaning weight was 72.25±0.32 kg. The calves were fed colostrum milked from their mothers half an hour after birth by using a bucket with a nipple. After the colostrum period, calves were fed with whole milk (Table 2) from the milking cows of the facility for 56 days for a total of 4 kg twice a day from the bucket until weaning. From the 4th day until weaning, starter feed, dry alfalfa grass and water were given freely to the calves during the liquid feeding period. In the study, the veterinary surgeon collected blood samples from the tail vein in non-anticoagulant tubes within the framework of the necessary rules after birth and at weaning. Blood samples were transferred to the laboratory as soon as possible. The blood samples were stored at -20 °C until analyzed.

As a result of the analyses, the effect of sex on the analysis of blood parameters at birth and weaning period in male and female Holstein calves in Mediterranean climatic conditions was found to be different at the level of P<0.037 for glucose value and P<0.059 for Gamma-Glutamyl Transferase GGT value. For Phosphorus (P) value, the interaction effect was found to be different at P<0.037 level. The GGT values of female calves were 34,88 Ul/l at birth and 26,85 Ul/l at weaning, while the values of male calves were 31.06 Ul/l at birth and 23,59 Ul/l at weaning. Glucose levels of female calves were 55,06 mg/dl at birth and 38.08 mg/dl at weaning in male calves was 9,80 mg/dl at birth and 7,73 mg/dL at weaning; in male calves it was 8,37 mg/dL at birth and 8,79 mg/dL at weaning.

Keywords: Mediterranean, sex, Holstein, calf, birth, weaning, blood parameters

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## Introduction

Holstein breed is the leading cattle breed in the world in terms of milk production. This is because this breed stands out with its high milk yield worldwide and although it has a low fat content of 3.5-4%, it can give between 9,000-12,000 liters of milk in a 305-day lactation period.<sup>1</sup> Although Holstein breed is grown in almost every region of the world, it is also preferred in hot and humid regions such as the Mediterranean with its very good yield. Calves of this breed also play an important role in herd continuity and calf loss rates vary between 2% and 30% depending on the conditions. For this reason, the performance results of calves under different conditions are important for the continuity of future production.

The majority of the studies on calves are focused on care and feeding. However, blood tests, which have been used in recent years, have gained importance by revealing potential risk factors in healthy individuals. Knowing the blood parameters of calves is extremely important in terms of monitoring their health status, evaluating their development and early diagnosis of potential problems. Blood parameters provide important information about the metabolic, immunological and nutritional status of calves.<sup>2–5</sup> The level of glucose in the blood indicates whether energy needs are being met. Low levels may be a sign of starvation or metabolic stress. Mineral and

vitamin levels are critical for growth and development. Deficiencies can lead to problems such as bone deformations, immune weakness and growth retardation.<sup>4,6</sup> Liver and Blood urea nitrogen (BUN), creatinine and enzyme levels provide information about the health of kidney functions.7 Blood pH levels and gas analyses are used to detect metabolic or respiratory imbalances (acidosis and alkalosis). Electrolyte levels (sodium, potassium, chlorine) are important to understand fluid loss due to diarrhoea or dehydration. It is also possible to evaluate the nutritional status and liver functions by using protein and albumin levels to understand whether the calf's growth and development continues in a healthy way. There are few studies7-9 (Tennant et al., 1974) on blood parameters in Holstein calves. In general, it is reported that the blood parameters of adult cows in Holstein breed are different from the blood parameter values of newborn calves.<sup>10,11</sup> Dubreuil and Lapierre<sup>12</sup> reported that there is no age-related change in Ca, P and Mg amounts in calves. Gökçe et al.13 reported that the differences between the total protein, albumin, urea, BUN, BHBA, NEFA, total cholesterol, ALT, AST, GGT, Ca, P and Mg values of Holstein calves were not statistically significant. Göncü et al.9 report that the differences in blood values between milk replacer groups were not statistically significant, except for Gamma Glutamyl Transferase (GGT) Ul/l. It is also stated that the interaction effect between the average values of total protein and cholesterol is

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important. Monitoring of blood parameters of calves is important for ensuring optimum growth and development and for future herd management decisions. For this reason, there is a need to determine the ideal values of blood values obtained under different conditions in calves and to determine blood values for herd management planning. Determination of blood parameters of male and female Holstein calves at birth and weaning period in Mediterranean climatic conditions provides important information for the evaluation of health status, development and adaptation of calves to environmental factors.

From this point of view, the aim of this study was to determine the blood parameters of male and female Holstein calves at birth and weaning period under Mediterranean climatic conditions.

## **Material method**

This research project received ethical approval from the Cukurova University Animal Experiments Local Ethics Committee on 20 May 2024, under decision number 2 in meeting 5. This study was conducted in Adana, located in the Mediterranean region between 37°0′ North latitude and 35°19′ East longitude. In the Mediterranean region, summers are hot and dry, winters are mild and rainy (Figure 1). While a milder climate is observed in the coastal regions, continental climate characteristics are also observed towards the north under the influence of the Taurus Mountains.

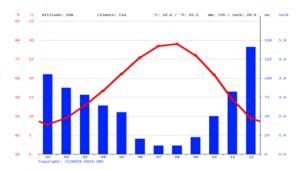


Figure I Adana province climate information (Climate.data.org).

The 54 blood samples of the study were taken from Holstein male calves born in October, November and December at the Çukurova University Faculty of Agriculture Research and Application Farm Dairy Cattle Farm. (The data used in this article was produced from project no. ZF2013BAP21) The average birth weight of the calves was  $37.52\pm0.42$  kg and the average weaning weight was  $72.25\pm0.32$  kg. The calves were fed colostrum milked from their mothers half an hour after birth by using a bucket with a nipple. After the colostrum period, calves were fed with whole milk (Table 1) from the milking cows of the facility for 56 days for a total of 4 kg twice a day from the bucket until weaning. From the 4th day until weaning, starter feed, dry alfalfa grass and water were given freely to the calves during the liquid feeding period. Milk dry matter, fat, protein, casein, lactose, urea levels were measured by milk analyzer (FT-120, Milkoscan, FOSS, Denmark).

Table	L.	Milk	compositio	n fed	to	calves
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Milk composition	%
Fat	3.78 <mark>±</mark> 0.23
Protein	3.71 <u>±</u> 0.22
Lactose	3.80±0.31
Organic Matter	12.14 <mark>±</mark> 0.12
Dry Matter	.7  <mark>±</mark> 0.09
Crude Ash	0.70 <mark>±</mark> 0.01

Calves are kept in individual pens opposite the birth pen for the first 3 days after birth. After the 3rd day, the calves are kept in fiberglass (width 106 cm, length 118 cm, height 140 cm) huts (Figure 2) until weaning.



Figure 2 Sheds where calves are kept until weaning.

Straw bedding material was used in the calf huts, and as the bedding material got wet, additional straw was thrown on it to provide a dry floor. Crude nutrient analyses (dry matter, crude fat, crude protein, crude ash) of calf starter feed and alfalfa hay used in calf feeding were performed according to AOAC<sup>14</sup> and NDF and ADF analyses were performed in Ankom Daisy Incubator using filter bag technique according to Van Soest et al.<sup>15</sup> The results of calf starter, calf growth and alfalfa hay chemical composition analyses are given in Table 2.

Chemical composition (%, based on dry matter)	Calf Starter	Alfalafa hay
Dry matter	92.38	92.72
Crude protein	18,12	11.75
Ether extract	4.2	0.82
Crude fiber	10.23	33.23
Ash	8	7.23

Blood samples were taken from the calves, which were the animal material of the study, twice, immediately after birth and at weaning. Blood samples were taken from the tail vein into anticoagulant-free tubes (10 ml, BD Vacutainer Systems, Plymouth, England) between 10:00-15:00 hours during the day by the veterinarian of the facility by observing the necessary rules and delivered to the laboratory as soon as possible. The sera obtained after centrifugation (Universal 320R, Hettich, Germany) at 4°C and 4000 rpm for 15 minutes were transferred to 1.5 ml ependorf tubes with protocol number. The blood samples were stored at -20°C until analysed. Ca concentration in sera was determined by using Arsenazo III method in the autoanalyser device (BS-120 Vet, Mindray, China) in the Feeds and Animal Nutrition Laboratory with the help of commercial kits.

Health observation of the calves was carried out twice a day by the calf carer and the researchers using a health scoring chart. When any health problem was detected in the calves, treatment was administered by the veterinarian. Blood samples taken from 3 calves due to health problems were excluded from the evaluation. The data obtained in the study were analyzed using Excel and SPSS programs.<sup>16</sup>

#### **Results and discussion**

In this study, the mean values of total protein, albumin, urea, BUN, BHBA, NEFA, total cholesterol, ALT, AST, GGT, Ca, P and Mg and the results of analysis of variance are given in Table 3.

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Table 3 Analyzes	results of the	blood parameter	of Holstein calves
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Blood parameters	Period	Mal	Male				Female					Significany level		
	Period	Ν	Mean	SEM	Min	Max	Ν	Mean	SEM	Min.	Max.	Sex	Time	Int.
Aspartate aminotransferase (AST, UI/I)	Birth	18	96,28	12,59	60	295	16	83,75	8,20	8	156		,572	,580
	Weaning	17	90,06	4,84	58	133	13	95,15	11,27	30	203	,547		
Gama Glutamyl	Birth	18	31,06	4,83	9	96	16	34,88	2,77	13	51	,590	,059	,724
Transferase (GGT, UI/I)	Weaning	17	23,59	3,08	7	48	13	26,85	4,98	12	80			
Cholesterol (mg/dl)	Birth	18	150,33	7,89	77	197	16	164,81	7,31	106	216	,115	,825	,898
	Weaning	17	150,00	8,07	87	206	13	157,08	11,44	92	220			
Glukoz (mg/dl) Birth Wear	Birth	18	53,67	4,88	24	86	16	55,06	4,95	38	97	,747	,037	,935
	Weaning	15	37,73	4,88	15	72	13	38,08	4,72	17	80			
Calcium	Birth	18	11,01	0,19	9,43	12,5	16	11,34	0,31	9,26	14,59	157	,355	,848
(Ca, mg/dl)	Weaning	17	10,79	0,18	9,13	11,9	13	11,14	0,13	10,08	11,81	,157		
Phosphorus	Birth	18	8,37	0,35	6,66	13,51	16	9,80	0,44	6,55	13,57	454	,454	,037
(P, mg/dl)	Weaning	17	8,79	0,45	3,52	11,78	13	7,73	0,44	5,12	10,57	,454		
Magnesium	Birth	18	2,74	0,35	1,52	7,89	16	2,55	0,09	2,12	3,28	570	,497	,881
(Mg, mg/dl)	Weaning	17	2,41	0,09	1,69	3,18	13	2,28	0,16	1,2	2,95	,579		
Total protein	Birth	18	8,26	0,73	5,54	19,89	16	8,11	0,29	5,53	9,53	,812	,352	,623
(TP, g/dl)	Weaning	17	7,11	0,30	5,46	9,41	13	7,24	0,37	4,92	9,38			
Albumin	Birth	18	3,45	0,03	3,13	3,75	16	3,51	0,02	3,41	3,75	,782	,578	,108
(ALB, g/dl)	Weaning	15	3,49	0,02	3,37	3,66	10	3,44	0,04	3,12	3,56			
Blood Urea Nitrogen	Birth	19	10,53	0,56	5,81	17,46	17	10,87	0,55	6,2	14,5	,107	257	254
BUN, mg/dl)	Weaning	16	9,07	0,84	3,9	13,82	П	12,43	1,31	5,6	20,28		,257	,254

As a result of the analysis, in the analysis of blood parameters at birth and weaning period in male and female Holstein calves in Mediterranean climate conditions, the effect of gender was found to be different at the level of P<0,037 for glucose value and P<0,059 for GGT value. For phosphorus value, the interaction effect was found to be different at P<0,037 level. The GGT values of female calves were 34,88 Ul/l at birth and 26,85 Ul/l at weaning, while the values of male calves were 31.06 Ul/l at birth and 23,59 Ul/l at weaning. Glucose levels of female calves were 55,06 mg/dl at birth and 38.08 mg/dl at weaning, and 53,67 mg/dl at birth and 37,73 mg/dl at weaning in male calves. Phosphorus value in female calves it was 8,37 mg/dL at birth and 8,79 mg/dL at weaning.

As a result of the analysis, while the effect of sex was found to be insignificant, the effect of period was found to be different at the level of P<0.037 for glucose value and P<0.059 for GGT value. Before birth, the calf meets its glucose requirement from the mother through the placenta. After birth, liver enzyme activities of calves are not yet fully developed. With increasing age, the gluconeogenesis (glucose production) function of the liver increases and the glucose level becomes more stable. The calf also starts to consume solid feed until weaning. Solid feed supports rumen development and causes changes in carbohydrate metabolism. This contributes to changes in glucose levels. In addition, regular milk intake after colostrum, which is not a source of glucose in calves, increases and stabilizes blood glucose levels. In calves, GGT is an enzyme and biochemical parameter used to evaluate liver function and biliary tract status. GGT levels in newborn calves are closely related to colostrum intake. Colostrum contains high amounts of GGT and this enzyme increases in the calf's bloodstream with colostrum consumption. Therefore, high GGT levels in the first days after birth are considered an indicator of adequate colostrum intake. Over time, as colostrum consumption decreases and the liver matures, GGT levels decrease and reach a normal balance. Therefore, differences tended to be significant between birth and weaning. Thompson and Pauli17 reported that serum GGT levels in the blood samples of calves receiving insufficient colostrum were 60 times higher than the GGT level in healthy adult cattle. It is reported that it takes about 5 weeks for this high GGT level to decrease to adult values. Another result is that the average GGT level observed in colostrum from six newly calved cows is 800 times higher than the average serum GGT level of the same cows. Therefore, it is highly likely that GGT will be high due to the simultaneous absorption of colostrum by the calves. Parish et al.18 reported that the GGT values 400 to 800 times higher in colostrum than in in serum from adult cattle. Feitosa et al. 2013 reported that GGT levels decrease as age progresses due to the renal filtration or biological degradation. Also, many researchers reported that GGT may be inferred from colostrum Ig transfer.<sup>19,20</sup> The effect of gender and period interaction for phosphorus value was found to be different at P<0.037 level. Phosphorus is critical for mineralization of bones and energy metabolism. After birth, calves become independent from placental phosphorus sources and their metabolism establishes a new balance. Phosphorus values are usually higher in newborn calves due to a rapid growth process. At 2 months of age, calves start to consume solid feed and rumen development is completed.<sup>21</sup> During this period, the phosphorus source changes and phosphorus metabolism in the rumen starts to be effective. After birth, calves become independent from placental phosphorus sources and their metabolism establishes a new balance. This may be one reason for changes in blood phosphorus levels. In the first days after birth, the high bioavailability of phosphorus through colostrum supports blood phosphorus levels.22 However, as colostrum consumption decreases and milk is replaced by solid feeds, age-related changes in blood phosphorus levels are observed. However, in this study, the

effect of sex and period inetraction was found to be significant. After birth, the levels of sex hormones (such as testosterone, estrogen) are quite low between male and female calves and these hormones have no significant effect on phosphorus metabolism. However, as they approach 2 months of age, hormonal regulations gradually begin to change and estrogen and testosterone can have indirect effects on the transport and storage of phosphorus in the bones. Male calves generally grow faster than females.<sup>22,23</sup> Therefore, they may require more phosphorus for bone development. This rapid growth may cause blood phosphorus levels to be slightly lower in male calves because phosphorus is used more rapidly for bone mineralization.<sup>24</sup> In the study, it is possible that the variations within the groups in terms of total protein, albumin, urea, BUN, BHBA, NEFA, total cholesterol, ALT, AST, Ca, P and Mg values in Holstein male calves during the liquid feeding period were effective in the statistically insignificant differences detected.

The mean values of total protein, albumin, urea, BUN, BHBA, NEFA, total cholesterol, ALT, AST, GGT, Ca, P and Mg in the blood samples are given schematically in Figure 3.

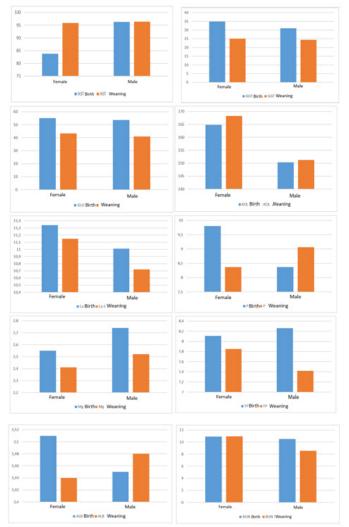


Figure 3 The male and female Holstein calves blood values at birth and weaning.

 $Eren^{25}$  reported Ca and P values as 10.18±0.57 mg/dl, 7.45±0.36 mg/dl and 8.70±0.37 mg/dl and 7.74±0.19 mg/dl in the control group

on the 4th, 8th, 15th and 29th days, respectively. On the 8th day, Ca and P values were reported as 9.87±0.56 mg/dl, 9.21±0.78 mg/ dl in the control group and 8.67±0.72 mg/dl, 8.57±0.40 mg/dl in the experimental group, respectively. On day 15, Ca and P values were reported as 9.33±0.73 mg/dl, 8.60±0.38 mg/dl in the control group and 11.08±0.31 mg/dl, 8.32±0.27 mg/dl in the experimental group, respectively. Eren<sup>25</sup> reported 4th, 8th and 15th day total protein values as 5.80±0.47 g/dl, 6.07±0.24 g/dl and 5.89±0.33 g/dl in the control group and 4.47±0.20 g/dl, 4.70±0.23 g/dl and 4.77±0.21 g/ dl in the experimental group, respectively. Albumin values were reported as 2.43±0.12 g/dl, 2.77±0.16 g/dl and 2.85±9.5 g/dl in the control group and 2.23±4.23 g/dl, 2.68±8.0 g/dl and 2.71±5.65 mg/ dl in the experimental group, respectively. Urea values were reported as 35.18±4.83 mg/dl, 25.48±4.93 mg/dl and 24.38±4.80 mg/dl in the control group and 36.83±4.13 mg/dl, 40.63±6.14 mg/dl and 21.22±2.25 mg/dl in the experimental group, respectively. Eren<sup>25</sup> reported that the values determined as a result of the experiment he conducted were within normal limits. Mohri et al.8 reported that calf blood biochemical values change with increasing age. Gustafsson and Palmquist<sup>26</sup> stated that especially feeding and sampling time affect BUN concentrations. Visek27 reported that blood ammonia concentration peaks at approximately 60 minutes after feeding, while BUN concentration is at its lowest level before feeding, reaches its highest level at approximately 2 hours after feeding and starts to decrease gradually until the 12th hour after feeding. Knowles et al.25 reported that albumin levels in calves were below the adult reference range until after nine days of age, but Egli and Blum<sup>29</sup> reported that albumin levels were within adult reference values from 28 days of birth. Zanker et al.30 reported that albumin is above the reference range after 60 days of age. Gökçe et al.<sup>13</sup> reported that total protein, albumin, urea, BUN, BHBA, NEFA, total cholesterol, ALT, AST, GGT, Ca, P and Mg values in Holstein male calves were 6.94±0.07 g/ dl, 2. 98±0.09 g/dl, 30.00±0.99 mg/dl, 14.01±0.46 mg/dl, 0.78±0.09 mmol/L, 0.39±0.05 mmol/L, 173.19±7.55 mg/dl, 28.49±1. 50 Ul/l, 85.05±2.64 Ul/l, 28.01±3.20 Ul/l, 8.73±0.12 mg/dl, 6.01±0.44 mg/ dl, 2.14±0.04 mg/dl. However, they concluded that the differences between the total protein, albumin, urea, BUN, BHBA, NEFA, total cholesterol, ALT, AST, GGT, Ca, P and Mg values of healthy Holstein calves in the months after birth were not statistically significant. Göncü et al.9 reported that the initial blood parameters of the groups were examined by gender, and it was determined that the differences between the milk replacer groups were not statistically significant except for GGT Ul/l. Also, when the mean values of total protein and cholesterol were examined, the interaction effect was found to be significant.

The results obtained in this study are similar to those of Eren<sup>25</sup> and Mohri et al.<sup>8</sup> that blood values increase with age. In addition, it is in agreement with the report of Egli and Blum<sup>29</sup> that the albumin value of calves is close to the adult age values, while Zanker et al.<sup>30</sup> differ from the reports of (2001). It is similar to the results of Gökçe et al.<sup>13</sup> and Göncü et al.<sup>9</sup>

## Conclusion

As a result of the analysis performed in this study, in the analysis of blood parameters of male and female Holstein calves at birth and weaning period in Mediterranean climatic conditions, the gender effect was found to be different at the level of P<0,037 for e Glucose value and P<0,059 for GGT value. For phosphorus value, the interaction effect was found to be different at P<0,037 level. The GGT values of female calves were 34,88 Ul/l at birth and 26,85 Ul/l at weaning, while the values of male calves were 31.06 Ul/l at birth and 23,59 Ul/l

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at weaning. Glucose levels of female calves were 55,06 mg/dl at birth and 38.08 mg/dl at weaning, and 53,67 mg/dl at birth and 37,73 mg/dl at weaning in male calves. Phosphorus value in female calves was 9,80 mg/dl at birth and 7,73 mg/dL at weaning; in male calves it was 8,37 mg/dL at birth and 8,79 mg/dL at weaning.

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## **Conflicts of interest**

The author declares no conflict of interest in writing the manuscript.

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