Acta Veterinaria (Beograd), Vol. 46. No.4, 203–210, 1996.

UDK 619:612.11

HEMATOLOGICAL, BIOCHEMICAL AND HORMONAL PROFILES OF FEMALE SAHIWAL COWS IN DIFFERENT PHYSIOLOGICAL STATES

F. ZAKIA, M.M. ASIF*, ZIA-UR-RAHMAN, Z. H. NAQVI, A. NAWAZ, M. MUSHTAQ-UL-HASSAN and F. KAUSAR

*Departement of Zoology, Government College, Faisalabad, Pakistan. *Department of Physiology & Pharmacology, University of Agriculture, Faisalabad-38040, Pakistan.

(Received,

The present study was undertaken to determine the effects of physiological state on hematological, biochemical and hormonal parameters in the blood in Sahiwal cows. The study was conducted on seven healthy Sahiwal cows i. e. young female calves (≤ 10 months), pregnant non-lactating (in the last trimester), lactating cattle (primiparous) and dry cows (dry not pregnant) at the livestock experimental station, University of Agricultural, Faisalabad. Ten ml of blood was collected from the jugular vein of the experimental animals in heparinized (0. 1 ml/ml) test tubes. The blood was divided into two parts, one of which was used for hematological parameters, while plasma was separated from the second part and stored in small aliguots at - 20^{0}C for biochemical and hormonal analyses. Significant differences were observed in total leukocytic count (TLC), total erythrocytic count (TEC), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Erythrocyte sedimentation rate (ESR) also differed significantly at one and 24 hour intervals. Neutrophils, lymphocytes and eosinophils were also significantly different between physiological states. Among the biochemical parameters, total plasma protein, globulin and urea were significantly different between different physiological groups. Both thyroxine (T4) and tri-iodothyronine (T3) also differed significantly in the four physiological states.

Key words: hematology, hormonal profiles, hemoglobin, Thyroxine

INTRODUCTION

The potential of the Sahiwal cow for milk and meat production has not been fully exploited in Pakistan. To meet the demand for milk and meat, more and more emphasis is being placed on improvement of the genetic potential of this species. In Pakistan, certain physiological (Zia-ur-Rahman et al., 1982) and biochemical

(Zia-ur-Rahman et al., 1993) profiles of Sahiwal heifers have been reported during summer months.

Stampfli et. al. (1981) described the results of analyses of blood samples taken between 7-9 hours from the jugular vein of 297 clinically healthy heifers 1-29 months old from different herds in central Switzerland. Variations with age were observed in 13 parameters and the highest values were obtained for RBC count and glucose. Gillani (1984) reported average values of 7.48 ± 1.98 million/mm^3, 11641.5 ± 1239.5 thousand/mm^3, 11.6 ± 2.80 g/dl and 35.35 ± 6.35% for RBC, WBC, Hb and PCV, respectively, in 200 buffaloes of different ages in different physiological states. Kumar and Sharma (1991) demonstrated that hemoglobin, cholesterol, glucose and phosphorus levels became significantly lower in the animals which did not become pregnant.

Majeed et al. (1983) reported serum protein (7.34 ± 0.06 g/dl), albumin (2.70 ± 0.04 g/dl) and globulin (4.55 ± 0.05 g/dl) concentrations in 93 buffalo-calves of either sex ranging from 1-30 months in age. Jindal and Ludri (1991) indicated that the concentration of T_3 is generally negatively correlated with milk yield in cows.

Very little work (Zia-ur-Rahman et al., 1980; 1982; 1993) has been done on the Sahiwal cow. Therefore the present study was designed to evaluate the blood picture of the Sahival cow in different physiological states, i. e. to determine different hematological, biochemical and hormonal parameters of apparently healthy calves, pregnant, lactating and dry Sahiwal cattle.

MATERIALS AND METHODS

The study was conducted on seven healthy Sahiwal cows in four phusiology states, i. e. young female calves, pregnant non-lactating caws, dry and lactating cattle at the livestock experimental station, University of Agriculture, Faisalabad. The calves were ≤ 10 months of age, the lactating cows were at their peak milk production (primiparous), pregnant cows were in the last trimester of pregnancy, and the dry cows were not pregnant. Ten ml of blood was collected from the jugular vein of these animals in heparinized (0.1 ml/ml) test tubes. Whole blood was divided into two parts, one was used for hematological parameters, while plasma was separated from the second part and stored in small aliquots at -20^{0}C for biochemical and hormonal analyses.

Total leukocytic count (TLC) and total erythrocytic count (TEC) were determined in a hemocytometer; hemoglobin (Hb) concentration was determined by the acid hematin method using Sahli's instrument; packed cell volume (PVC) was determined using microhematocrit tubes; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the results for RBC, Hb, and PCV. Erythrocyte sedimentation rate (ESR) was determined using Wintrobe's graduated hematocrit tube (Benjamin, 1978). The differential leukocytic count (DLC) was obtained by the method described by Kolmer et al. (1961).

Total plasma protein was determined on the Boush & Lomb refractometer (Coles, 1974). Plasma albumin (g/dl) and plasma urea (mg/dl) were estimated by using commercially available Randox Kits. Globulin concentration (g/dl) of the plasma was obtained by subtracting the amount of albumin from that of total protein.

Tri-iodothyronine (T_3) and thyroxine (T_4) concentrations of serum were estimated by an enzyme immuno kit (Immunitech Corp. Boston M. A. 02134).

Analysis of variance (Steel and Torrie, 1980) was used to find out if there were significant effects of physiological state on the hematologicala, biochemical and hormonal parameters. Duncan's Multiple Range Test (Duncan, 1955) was used to compare the mean values among groups.

RESULTS AND DISCUSSION

The present study demonstrated a significantly lower TLC (10.56 ± 0.75 $10^3/\mu l$) in calves than in dry cows (Table 1). The TLC in adult dry cows was 17.84 ± 1.43 10^3 / μl (Table 1) which was much higher than the values noted in adult animals by Reda (1951). - 10.60 thousand/mm^3; Bhalla and Senger (1964). - 9.20 thousand/mm^3; Hussain and Ahmad (1968).-7.50 thousand/mm^3; Vagher et al. (1973). - 8.87 thousand/mm^3; Gillani (1984). - 11.64 thousand/mm^3 and Zia-ur-Rahman et al. (1993)- 10.88 thousand/mm^3 in heifers. However, ,some investigators (Reda, 1951; Bhalla and Senger, 1964) found a higher TLC for calves than for adults. A higher TLC might be associated with conditions that resulted in the release of epinephrine during collection of blood. The TLC in lactating cows was 11.64 ± 0.69 10^3 / ml (Table 1). The values for lactating and nonlactating cows were different from the values for buffaloes reported by Rizvi (1973). The general trend for TLC counts is high at birth in calves and growing animals, followed by a gradual reduction with advancing age (Wittwer and Bohmwald, 1974; Schalm and Jain, 1984).

Table 1. Mean total leukocytic counts (TLC) and differential leukocytic count (DLC) of sahiwal cattle in different physiological states.

Parameters	Physiological state			
	Calves	Pregnant	Milking	Dry
TLC (10^3 / μl)	10.56b ± 0.75	15.46ab ± 1.86	11.64b ± 0.69	17.84a ± 1.43
Neutrophil (%)	31.00b ± 2.94	47.29a ± 6.80	34.43b ± 2.22	37.57ab ± 1.70
Lymphocyte (%)	65.29a ± 2.41	55.57ab ± 2.04	58.71a ± 3.03	41.14b ± 10.08
Eosinophil (%)	0.57b ± 0.22	5.00ab ± 1.16	3.43ab ± 2.87	6.57a ± 2.87
Monocyte (%)	1.86 ± 1.07	1.00 ± 0.47	1.29 ± 0.49	1.43 ± 0.98
Basophil (%)	1.14 ± 0.69	0.86 ± 0.69	2.71 ± 0.90	4.00 ± 1.73

a, b: Similar letters in one row indicate values are not significantly different from each other at $p > 0.05$.

The neutrophil values differed significantly between different physiological states of thase cows. The lowest percentage (31.00 ± 2.94%) of neutrophils was

observed in calves while the highest (47.29 ± 6.80%) was observed in the pregnant cows. Thus, the relative neutrophil count increases with advancing age. The lymphocyte values estimated in the present study were highest (65.29 ± 2.41%) in the calves and lowest (41.14 ± 10.08%) in the dry animals (Table 1). This decrease in the relative lymphocyte count is to be anticipated in response to stress in dry cattle. In the present study the relative number of lymphocytes was significantly higher in calves than in the milking and dry cows. The lymphocyte count decreases with advancing age (Schalm and Jain, 1984).

The TEC in calves was 8.59 ± 1.37 and it was 8.52 ± 0.24 10^6 / μl in dry animals (Table 2). Similar results have been reported by Reda (1951) - 9.17 million/mm^3; Bhalla and Senger (1964) - 9.5 million/mm^3; Mastrangelo (1971). - 8.4-13.5 million/mm^3; Vagher et al. (1973) - 9.05 million/mm^3 and Gillani (1984) - 7.48 million/mm^3 in buffalo calves while the present study concerns cattle. The value observed by Mastrangelo (1971) was confirmed with the present value for TEC in calves. The TEC observed in the pregnant cows was 7.51 ± 0.42 $10^6/\mu$l. Schalm and Jain (1984) reported that TEC increases slightly as pregnancy advances. The slightly higher TEC and PCV in pregnant Sahiwal cattle may be due to supplementation with protein and meat meal concentrate in the normal diet during late pregnancy (Rowlands et al., 1974; Picard, 1977; Sykes, 1977). The mean red blood cell (TEC) count observed in milking cattle was 5.76 ± 0.38 10^6/ml which was significantly lower than in calves and dry cows. Schalm and Jain (1984) reported that lactation-related changes may be irregular and vary from herd to herd. Generally non-lactating cows have higher TEC values than lactating cows. This decline in TEC number in lactating cows may be related to milk yield (Hewett, 1974). High milk producers tend to develop anemia more frequently than low producers, particularly during the winter (Noonan, 1978).

Table 2. Mean total erythrocytic count (TEC), hemoglobin (Hb), packed cell volume (PCV), erythrocytic indices (MCV, MCH, MCHC) and erythrocyte sedimentation rate (ESR) of sahiwal cattle in different physiological states.

Parameters	Physiological state			
	Calves	Pregnant	Milking	Dry
TLC (10^6 / μl)	8.59a ± 1.37	7.51ab ± 0.42	5.76b ± 0.38	8.52a ± 0.24
Hb (g/dl)	12.39 ± 0.31	12.29 ± 0.86	12.02 ± 1.06	13.31 ± 0.50
PCV (%)	33.57c ± 0.74	36.78b ± 0.56	39.79a ± 0.81	32.20c ± 0.92
MCV (fl)	44.49b ± 6.82	49.86b ± 3.26	69.90 ± 3.16	37.96b ± 1.47
MCH (pg)	16.42 ± 2.57	16.61 ± 1.53	20.98 ± 1.71	15.64 ± 0.47
MCHC (g/dl	36.95ab ± 0.94	33.34b ± 2.11	30.20b + 2.67	41.64a ± 2.49
ESR (mm/h)	0.00c ± 0.00	0.00c ± 0.00	0.86a ± 0.45	1.00b ± 0.58
ESR (mm/4h)	0.57 ± 0.20	2.18 ± 1.20	1.48 ± 0.90	2.00 ± 1.12
ESR (mm/24h)	4.50b ± 0.71	4.14b ± 0.62	6.43a ± 0.69	4.57b ± 1.43

a, b, c: Similar letters in one row indicate values which are not significantly different from each other at $p > 0.05$.
MCV: Mean corpuscular volume.
MCH: Mean corpuscular hemoglobin.
MCHC: Mean corpuscular hemoglobin concentration.

Non-significant differences in PCV values were observed between calves and dry cows (Table 2). These findings relate to TEC, which did not differ significantly between these two groups. However, the highest PCV (39.79 ± 0.81%) was observed in milking cattle whereas TEC was the lowest in these animals. The low erythrocyte count and high PCV in these animals may be attributed to an increase in cell size (Zia-ur-Rahman et al., 1993). The MCHC is a more valuable measurement than MCH, because in anemia in which the MCH is not altered proportionally with MCV the lack of correlation is indicated by a change in the MCHC.

The ESR was observed at three different times during the study i.e. after 1, 4 and 24 hours in the four groups. The results obtained suggest that measurements of ESR at 4 hours were not suitable since settling of cells occurs only to a negligible extent during this interval. Similar observations on the timing of measurements of ESR were reported by Zia-ur-Rahman et al. 1980) while measuring the adaptability of cross-bred dairy animals during the summer season. After 24 hours the maximum sedimentation (6.43 ± 0.69 mm) took place in the milking cattle while the lowest value (4.14 ± 0.62) was observed in the pregnant animals. Physiological features such as changes in water balance and pregnancy may temporarily elevate plasma fibrinogen. Fibrinogen or total plasma protein levels are perhaps the most important factor regulating ESR.

Table 3. Mean plasma biochemical and hormonal profiles in sahiwal cattle in different physiological states.

Parameters	Physiological state			
	Calves	Pregnant	Milking	Dry
Protein (g/dl)	7.57b ± 0.55	9.30a ± 1.22	9.79a ± 0.65	9.87a ± 0.50
Albumin (g/dl)	2.67 ± 0.35	2.49 ± 0.24	2.14 ± 0.43	2.59 ± 0.11
Globulin (g/dl)	4.90b ± 0.82	6.92a ± 0.85	7.02a ± 1.23	7.56a ± 0.77
Urea (mg/dl)	6.66b ± 0.70	12.12ab ± 0.86	6.78b ± 1.95	17.43a ± 3.05
Thyroxine (T_4 μg/dl)	4.40b ± 0.21	4.50b ± 0.28	5.50a ± 0.29	2.50 ± 0.10
Tri-iodo Thyronine (T_3 μg/dl)	1.88a ± 0.20	1.75a ± 0.18	0.70b + 0.12	0.98b ± 0.25

a, b: Similar letters i;n one row indicate values which are not significantly different from each other at $p > 0.05$.

The plasma proteins represent a complex mixture containing many components which differ in properties and functions. The amount of protein varied significantly among the four physiological states. The lowest protein concentrations (7.75 ± 0.55 g/dl) were present in the calves while the highest level of protein (9.87 ± 0.50 g/dl) was found in dry cows (Table 3). Majeed et al. (1983) reported protein contents in the serum of calves of 7.34 ± 0.06 g/100 ml which is well in accord with the present values. Hamza and El-Abdin (1976) determined the protein concentration in the lactating buffalo which was 7.56 g/100 ml. This value is much lower than the value (9.79 ± 0.65 g/dl) observed for milking cattle (Table 2). The striking increase in total protein was due to a significant increase in

globulin, which contributed towards the increase in total proteins in pregnant, dry and milking animals compared to calves. An alteration in protein fractions changed the ratio A/G. A significantly higher level of plasma urea was observed in dry animals. This might be due either to increased deamination of amino acids or increased protein intake (Sykes, 1977). Urea concentrations did not differ between calves and milking cattle, and were the lowest in these groups (Table 3). This is indicative of low net protein degradation by these animals.

Dry cows of the present study had the lowest concentration (2.50 ± 0.70 mg/dl) of thyroxine, while the highest value (5.50 ± 0.29 mg/dl) occurred in lactating cattle (Table 3). Henneman et al. (1955) showed that lactation resulted in increased demends upon the thyroid gland. Magoub and Johnson (1977) stated that plasma thyroxine level and secretion rate were higher in dry than high producing cows. Pichaicharnarong et al. (1982) found that serum thyroxine increases during pregnancy with a pronounced fall at full term and a return to the non-pregnant level one month postpartum. Reana et al. (1989) studied serum thyroxine concentration in Nili-Ravi buffaloes. The serum level of thyroxine (T_4) measured by ELISA revealed a significantly higher mean 7.86 ± 3.45 mg/dl in five heifers than in 10 lactating (3.30 ± 1.40 mg/dl) and 20 pregnant buffaloes. The serum T_4 was similar during lactation, early pregnancy (4.20 ± 1.3 mg/dl) and late pregnancy (2.20 ± 0.60 mg/dl) in buffaloes. Milk yield and serum T_4 concentration did not show any relation. Jindal and Ludri (1991) indicated that the concentration of T_3 is generally negatively correlated with milk yield in cows.

In brief, significant differences were observed in total leukocyte count (TLC), total erythrocyte count (TEC), packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Erythrocyte sedimentation rate (ESR) also differed significantly at one and 24 hour intervals. The relative contributions of neutrophils, lymphocytes and eosinophils significantly differed among the four physiological states. Among the biochemical parameters, total plasma protein, globulin and urea were significantly different between different physiological groups.Both thyroxine (T_4) and triiodothyronine (T_3) levels also differed significantly among the four physiological states.

REFERENCES

1. *Benjamin, M. M.* 1978. Outline of Veterinary Clinical Pathology. 3rd *Edit. Kalyani. New Delhi-Ludhiana.*

2. *Bhalla, R. C. and Senger.* 1964. Blood picture of buffalo calves in early postnatal life. *Indian Vet. J. 41*, 38-41.

3. *Coles, H. H.* 1974. Veterinary Clinical Pathology. 14th Ed. *Saunder Co. Philadephia, pp.* 123-150, 156.

4. *Duncan, D. B.* 1955. Multiple Range and Multiple F. test. *Biometrics, 11.* 1-42.

5. *Gillani, S. W. S.* 1984. Study on the normal hematology and biochemistry of blood of buffaloes. M. Sc. *Thesis, Dept, Vet. Pathology, C. V. S., Univ. Agri; Faisalabad.*

6. *Hamza, S. M. and El-Abdin, Y. Z.* 1976. Studies on some biochemical constituents and enzymes in the serum of the normal nonpregnant dairy Egyptian buffaloes. *J. Egyptian Vet. Med. Assoc. 36 (1)*, 169.

7. *Henneman, H. A., Reineke, E. P. and Griffin, S. A.* 1955. The thyroid secretion rate of sheep as affected by season, age, breed, pregnancy and lactation. *J. Anim.Sci. 14 (2)*, 419-434.

8. *Hewett, D.* 1974. On the causes and effects of variations in the profile of Swedish dairy cattle. *Acta. Vet. Scand. Supple. 50*, 1.

9. *Hussain, A. S. K. and Ahmed, A.* 1968. Hematological values for normal buffalo in East Pakistan, University dairy farms. Pak. *J. Vet. Sci. 2 (3)*, 126-133.

10. *Jindal, S. K. and Ludri, R. S.* 1991. Circulating thyroxine (T_4) and triiodothyronine (T_4) levels in lactation crossbred cows and buffaloes as affected by stage of lactation & time of sampling. National Dairy Research Institute, Kernal, Haryana, India. *Int. J. Anim. Sci 6 (2)*, 122-127.

11. *Kolmer, J. A., Spulding, E. H. and Robinson, H. W.* 1961. Approved laboratory technique. 5th Edit. p. 75.

12. *Kumar, S. and Sharma, M. C.* 1991. Level of hemoglobin and certain serum biochemical constituents in rural cows during fertile & non-fertile oestrus. *Indian Vet. Res. Ins. Izat nagar.*

13. *Magoub A. B. and Johnson, H. D.* 1977. Estimation of thyroid function in relation to the milk production by measurement of plasma thyroxine and thyroxine turnover. *J. Dairy Sci. 60*, 106.

14. *Majeed, M. A., Manawar, S., Ahmed, A., Toor, M. A. and Zia-Ur-Rehman.* 1983. Effects of sex and age on the serum proteinogram in growing buffalo-calves. *Pak. Vet. J. 3 (2)*, 65-69.

15. *Masterangelo, P.* 1971. Study of blood cell hemoglobin concentration in domestic animals. Note 5. buffaloes. *Acta. Med. Vet. Napoli. 17*, 115-122. 5509.

16. *Noonan, T. R.* 1978. Effects of age, season and reproductive activity of hemogram of female Hereford cattle. *Am. J. Vet. Res. 39*, 433.

17. *Picard, D. W.* 1977. Calcium, magnesium and phosphorus in lactation and dry period. *British Soc. Anim. Prod. (1)*, 49-52.

18. *Pichaicharnarong, A., Lopyetjra, P., Chairyabutr, N., Usana Korn Kuls and Djurdjević, D. J.* 1982. Thyroid activities of non pregnant postpartum and new born Swamp buffaloes. *J. Agri. Sci. 98 (3)*, 483-486.

19. *Raana, R., Noureen, A., Ali, C. S. and Nawaz, M.* 1989. Serum thyroxine concentration in Nili-Ravi buffaloes. *Pak. Vet. J. 9 (4)*, 150-152.

20. Reda, H. 1951. Hematological examination of the normal blood of Egyptian buffaloes. *Am. J. Vet. Res. 12*, 23,25.

21. *Rizvi, S. A. R.* 1973. Study on the normal blood picture of buffaloes in Lahore. *M.Sc thesis, Dept. Vet. Pathology, C. V. S., Univ. Agri., Faisalabad.*

22. *Rowlands, G. J., Payne, J. M., Dew, S. M. and Manston, R.* 1974. Individuality and heritability of composition of values with particular reference to the selecltion of stock with improved growth potential. *J. Agri. Sci. 82*, 473-481.

23. *Schalm, O. W. amd Jain, N. C.* 1984. Veterinary hematology. 4th Edit. Balliere, Tindall and Cassell, *London. pp*, 178-206.

24. *Stampfli, G., Stirnimann, J. and Kupfer, U.* 1981. Influence of stage of lactation and pregnancy on hematological values in the dairy cow. *Schweizer Archiv fur Tierheilkunde, 123 (4)*, 189-205.

25. *Steel, R. G. D. and Torrie, J. H.* 1980. Principles and procedures of statistical analysis. 2nd Ed. *McGraw Hill, Kogakusha Ltd., Tokyo, Japan.*

26. *Sykes, A. R.* 1977. An assessment of the value of plasma nitrogen and albumin concentration as monitors of the protein status of sheep. British Soc. *Anim. Prod. 1*, 145-154.

27. *Vagher, J. R., Bearson, B., Blatt, S. and Kage, M.* 1973. Biochemical and hematological values in male holstein friesian and calves. *Amer. J. Vet. Res. 34*, 273.

28. *Wittwer, F. and Bohmwald, H. 1974. Leukocyte count in normal female Frisian cattle of various ages in the valdivia area. Chile. Arch. Med. Vet. 6*, 32.

29. *Zia-ur-Rehman, Gillani, A. H., Ahmad, M. D. and Ali, C. S.* 1980. Adaptation of crossbred dairy heifers to summer stress as measured by erythrocyte sedimentation rate. *J. Anim. Sci. Pakistan. 11 (3-4),* 22-27.

30. *Zia-ur-Rehman, Gillani, A. H., Ahmad, M. D. and Ali, C. S.* 1982. Adaptability of cross-bred dairy heifers to summer season measured by rectal temperature, pulse rate and respiration rate. *Rev. Elev. Med. Vet. Pays. Trop. 35* (1), 63-68.

31. *Zia-ur-Rehman, Khaliq, T., Javed, I., Hur, G. and Ali, L.* 1993. Comparative hematological and serum biochemical values of purebred and cross-bred heifers. *Pak. Vet. J. 13 (3),* 110-113.

NEKI HEMATOLOŠKI I BIOHEMIJSKI PARAMETRI I STATUS POJEDINIH HORMONA KOD RAZLIČITIH FIZIOLOŠKIH STANJA U KRAVA RASE SAHIWAL

F. ZAKIA, M. M. ASIF, ZIA-UR-RAHMAN, Z. H.NAQVI, A. NAWAZ, M. MUSTAQ-UL-HASSAN I F. KAUSAR

SADRŽAJ

U radu je prikazan uticaj fiziološkog stanja hematološke i biohemijske parametre i na status nekih hormona kod krava rase sahiwal i to kod ženske teladi uzrasta do 10 meseci, zasušenih krava u poslednjem trimestru steonosti, prvotelkinja u laktaciji i jalovih zasušenih krava. Analizom sledećih hematoloških parametara: broj eritrocita, broj leukocita, hematokrit (PCV), prosečna zapremina eritrocita (MCV), prosečni sadržaj hemoglobina u eritrocitima (MCHC) i sedimentacija posle jednog i 24 sata ustanovljene su statistički značajne razlike između ispitanih grupa. Odstupanja vrednosti ukupnih proteina krvne plazme, globulina, ureje, kao i koncentracije tiroksina (T4) i tri-jodtironina (T3) između grla u različitom fiziološkom statusu takođe su bila signifikantna.