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EFFECT OF DIFFERENT DIETARY ENERGY LEVELS, SEASON AND AGE ON HEMATOLOGICAL INDICES AND SERUM ELECTROLYTES IN GROWING BUFFALO HEIFERS

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ABSTRACT

Twenty one buffalo heifers of one year age and similar body weight were divided into three groups, A, B and C. Three total mixed rations (TMR) with different energy levels were randomly allotted to these groups. Ration B contained 100% NRC energy recommendations, (control) while ration A and ration C contained 80% and 120% energy. These rations were fed for a period of 18 months. Blood sampling was done at monthly intervals to determine the hematological indices and serum electrolytes. Data were analyzed using mixed model least squares and maximum likelihood computer program (ISMLMW). Energy levels had significant effect (P<0.05) on blood coagulation time. During winter, there was marked increase in blood coagulation time, (6.75 minutes) while, it was significantly decreased during summer. These values were higher (P<0.05) during post pubertal age. A significant increase in erythrocyte count was found during spring. The erythrocyte count during young age was lower (P<0.05) as compared to post pubertal stage. Energy levels had no effect on total leukocyte count. Erythrocyte Sedimentation Rate (ESR) values were significantly (P<0.01) affected by energy levels with maximum value in ration B. Season and age also had effect on ESR. PCV values were highest (P<0.05) on medium energy ration and lower (P<0.01) during younger age. Higher levels (P<0.05) of hemoglobin were found in group of buffaloes fed high energy ration. Season had no effect whereas, concentration was lower at pre-pubertal age. A significant increase in sodium concentration was recorded during post pubertal age as compared to pre- pubertal age (P<0.01). Potassium ion concentration was significantly affected by different energy levels in feed (P<0.05) with highest value in ration C. Season and age had also significant effect on potassium concentration (P<0.01). The chloride concentration was significantly (P<0.01) affected by dietary energy levels and season. Highest values of calcium concentration were recorded during spring and post pubertal stage as compared to pre pubertal stage (P<0.05).

Key words: Buffalo heifers, Blood coagulation time, Erythrocyte sedimentation rate (ESR), Packed cell volume (PCV), Hemoglobin concentration (Hb), and Total leukocyte count, (TLC).

INTRODUCTION

Buffalo is an important dairy animal in Pakistan with a population of 29.2 million (Anonymous, 2006) and is increasing at the rate of 5% annually. It contributes about 65% of total national milk production and more than 50 % beef production in the country. As the animal is restricted to the under developed countries, there is a serious constraint to buffalo production with low reproductive efficiency (Mahadevan, 1978). Climate influences cyclicity, estrus expression and conception rates through temperature, humidity and rain fall, (Ahmed *et al.* 1982), the effects of latter are mediated by the availability and quality of feed, (Lundstorm *et al*. 1982). Study of different hematological constituents of blood is of great importance during disease conditions, stress, immunity etc. They also have direct clinical application for diagnostic purposes. These parameters are influenced by environmental factors which ultimately affect the productive and reproductive performance of animals.

Keeping in view the importance and variation of these biochemical parameters under different conditions, a study was planned to see the effect of dietary energy levels and the influence of season and age of animals on these indices in growing buffalo heifers.

MATERIALS AND METHODS

Twenty-one buffalo heifers of one-year age were maintained at Nutrition Section of Livestock Production Research institute Bahadar Nagar, Okara under similar feeding and management conditions. Three months period was provided to condition the animals for handling and adaptation and randomly divided into three groups A, B, and C. Bio-metric data of each heifer was obtained from the farm records which included brand no, date of birth, and health status of the animals. Three rations of varying levels of metabolizable energy (ME) were

formulated. Ration A contained 80% of ME of NRC (1978) recommendations, Ration B had 100% while ration C had 120% ME. The composition of ration is presented in tables 1. Weighed quantities of respective rations were provided to the heifers in the morning on individual feeding basis in separate mangers. In the evening when they had finished ration they were let loose and had free access to water. The trial was continued for a period of 18 months.

Blood samples were taken from juggler vein on monthly interval. Blood was collected and used for various hematological parameters. Blood coagulation time was recorded by the method described by Rowsell, (1963). RBC count, ESR and TLC were determined by using the techniques as described by Benjamin, (1978). PCV was obtained by the micro-hematocrit method as described by Jain (1986). Hemoglobin was determined by using Sahli`s apparatus (Sastry, 1983). Serum sodium and potassium were estimated with the help of Corning-405 flame-photometer. Serum chloride was determined by the kit from RANDOX Laboratories (Fried et al.1972). Calcium concentration was determined using the technique of atomic absorption as described by Hwang and Sandnato, (1969).

Data on the above parameters was arranged according to season e.g. summer (May, June, July and August), autumn (September and October) winter (November- Feb.) Spring (March and April). Similarly effect of age (Pre-pubertal and post-pubertal) was also studied. Data was statistically analyzed according to Harvey (1980) by using mixed model least squares and maximum likelihood computer programmed (ISMLMW) PC-1 Version.

Table 1: Composition of rations A, B and C, with low energy level (80%), medium energy level, (100%) and high energy level, (120%) of NRC recommendation

Ingredients	Percentage (%)			Crude Protein (%)			Metabolizable Energy (Mcal/Kg)		
	Rations								
	A	B	C	A	B	C	A	B	C
Cotton seed cake	38.00	15.0	9.00	8.75	3.46	2.11	87.02	34.35	20.61
Maize gluten 60 %	01.00	6.0	10.00	0.60	3.88	6.47	3.42	20.52	33.42
Maize grain	01.00	5.0	20.00	0.09	0.49	1.96	3.58	17.90	71.60
Wheat bran	01.00	23.0	5.75	0.15	3.44	0.86	2.57	59.11	14.78
Wheat straw	42.00	24.0	14.00	1.09	0.62	0.36	65.00	37.20	21.70
Molasses	12.25	15.0	20.00	0.37	0.46	0.61	34.92	43.65	58.20
Rice polish	01.00	8.0	15.00	0.12	0.99	1.85	3.25	26.00	48.75
Urea	01.50	0.5	0.25	3.39	1.36	0.69	0.00	0.00	0.00
Vegetable oil	0.25	1.5	4.00	0.00	0.00	0.00	1.82	10.95	29.20
Mineral mixture	02.00	20.0	2.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	100.0	100.0	100.0	14.56	14.70	14.91	201.58	249.68	298.26

RESULTS AND DISCUSSION

Blood coagulation time: Energy levels in the diet had significant effect on blood coagulation time ($P<0.05$). It was greater ($P<0.05$) in group of buffalo heifers, kept on high plan of nutrition as compared to those maintained on medium and low energy plan of nutrition (table 2). During winter season, there was marked increase in blood coagulation time while, it was significantly decreased during summer ($P<0.01$). These results are in agreement with the findings of Majeed *et al.* (1985) who reported that average coagulation time during summer season was 4.95 ± 0.21 minutes and was affected by the season of year. Blood coagulation time was significantly ($P<0.05$) increased after the attainment of puberty (table 2). Majeed *et al.* (1985) observed that growing age affected the blood clotting time: in yearlings values were 4.05 ± 0.28 minutes, while, Adval and Gangwa, (1971) reported very high blood coagulation time of 11 minutes and 13 seconds recorded in the Indian calves.

The erythrocyte count: The erythrocyte count was not affected by the level of energy in the ration. Season of the year however, had significant effect on erythrocyte count. The highest values were found during spring, whereas, lowest count was noticed during autumn and the difference was significant ($P<0.01$). Siddiqi and Ahmed (1972) recorded the higher erythrocyte count in winter than in spring and summer. Anwar and Chaudari (1984) came with the conclusion different from the present study and reported that two extremes of weather did not affect the overall average of erythrocyte count among the buffalo calves of either sex. The age of animals showed non-significant variations.

Erythrocyte sedimentation rate (ESR): Significantly higher values for erythrocyte sedimentation rate (ESR) were observed in group of buffaloes fed on medium

energy level (P<0.01), while, these values were decreased in buffaloes kept on low energy ration. Variations in ESR values due to seasons of the year were also highly significant (P<0.01) with highest values observed during autumn, than other seasons. Rizvi (1973) reported higher value of ESR during winter compared to other seasons. Age of animals also affected the ESR value in heifers. There was marked increase in ESR values after the attainment of puberty. Patil *et al*. (1992) stated that erythrocyte sedimentation rate in newborn buffaloes was very low but later on it increased as the age advances. The inhibitory factors for ESR present in plasma and RBC might be disappearing with advancement of age. The exact mechanism is not known yet.

Packed cell volume: PCV values were highest in group of buffalo heifers offered medium energy ration, while lowest values were recorded in-group of buffaloes fed on low energy ration. These differences are highly significant (P<0.01). Similarly, seasonal changes also affected the PCV values (P<0.01). An increasing trend of PCV was seen during winter, whereas, these decreased during hot days. These findings are supported by the findings of Gangwar *et al*. (1984). However, Sulong *et al.* (1980) reported higher values during summer. The packed cell volume was lower during young age and it increased as age increased. Kumar *et al*. (1990) observed that haematocrit value showed a marked decrease in one-month-old calves as compared to day old and other ages. Patil *et al.* (1992) also noted the similar results and concluded that higher erythrocyte value was responsible for increased PCV value in growing buffalo heifers as compared to adult buffaloes. This may be due to high basal metabolic rate, which increased the rate of erythropoises resulting in higher number of erythrocytes.

Hemoglobin: Higher levels of hemoglobin were found in group of buffaloes fed on high energy levels, while these levels were lower in buffalo heifers of group A (P<0.05). Season of the year or age of buffalo heifers had no effect on hemoglobin concentration. Siddiqi and Ahmed (1972) found non-significant difference between summer spring and winter. However, Anwar and Chaudhary (1984) and Majeed *et al.* (1985) reported that significantly higher values of hemoglobin were recorded in summer than winter in adult buffaloes.

Hemoglobin concentration showed variation during different stages of age with higher values at post-pubertal stage. Kumar *et al*. (1990) reported that hemoglobin concentration was found to be higher at birth, with overall mean value of 12.76±0.61g/dl. Patil *et al*. (1992) found that the hemoglobin content in heifers was significantly higher than in non-pregnant and pregnant buffaloes. The higher Hb content in heifers was as per the physiological demand and endocrinological influences during growth period. Gangwar *et al.* (1984) reported that the high concentration of hemoglobin, PCV and RBC were the desirable physiological characteristics for the efficient transport of oxygen and carbon dioxide.

Total leukocyte count (TLC): Non-significant difference in the values of TLC was found in the buffalo heifers received different energy diets. The season revealed the significant differences in the values of leukocytes. Lower total leukocyte count was observed in autumn, while higher count was found during spring. Majeed *et al.* (1985) found that total leukocyte count was relatively, higher in summer than other seasons. Siddiqi and Ahmed (1972) and Anwar and Chaudhary (1984) reported that significantly higher values of total leukocyte count were recorded in summer in buffalo. This may be due to reduced blood volume due to dehydration during summer. TLC also increased with advancement of age which is supported by the findings of Thakar (1983); Majeed *et al.* (1985) and Kumar *et al.* (1990). They reported that average total leukocyte count showed an increasing trend with increase in age. The above results indicate that season of year and the age of animals more affect on hematological parameters while the dietary energy levels had lesser effect.

Electrolytes

Sodium: Sodium concentration was not affected by different energy levels. Significant seasonal variations in sodium concentration have been observed. The marked increase was seen in spring while, decrease during summer. Sodium concentration was also affected by age. Greater sodium concentration was recorded after the attainment of puberty. Difference among the values was significant (P<0.0.1). Gangwar *et al*. (1984) claimed better conception rate at high sodium levels in different developmental stages of buffalo heifers. However, Kumar et al. (1992) observed the significantly high plasma sodium concentration in postnatal calves. It was subsequently declined to basal level of 130.82 ± 0.01 mEq / L in young calves. Sodium ion concentration in pre-pubertal, pubertal and sexually mature heifers did not exhibit any noticeable difference.

Potassium: Potassium ion concentration was highest in buffalo group fed on medium energy diet, whereas, the values were lowest in blood of buffalo heifers maintained on low energy ration (P<0.05). Seasonal variations demonstrated the highly significant effects on potassium concentration during the year. Summer potassium values were the highest (4.84 ±0.034 mEq/L) as compared to those of spring values, (5.54 ±0.061 mEq/L). Majeed *et al.* (1985) found that extremes of weather had a significant effect on serum potassium level. Potassium concentration was observed to vary with age. It was highest at post-pubertal stage, while, lower at pre-pubertal stage. Kumar *et al*. (1992) also observed that plasma potassium level was higher in mature group of

buffalo heifers as compared to other developmental stages.

Table 2: Mean ± SE of hematological parameters in different groups of buffalo heifers

Parameters	Blood Clotting Time (minutes)	RBC (Million / cu. mm)	ESR (mm/hr)	PCV (%)	Hb (g/dl)	TLC (Thousands/ cu.mm)
Energy levels						
Low	5.89±0.05[a]	5.83±0.09	63.32±1.31[b]	32.81±0.32[c]	11.30±0.25[c]	12.62±0.22
Medium	5.65± 0.052[c]	5.87±0.091	70.48±1.33[a]	35.79±0.32[a]	11.75±0.25[b]	12.87±0.23
High	5.66±0.053[b]	6.02±0.093	61.21±1.35[c]	34.49±0.33[b]	12.19±0.26[a]	12.83±0.23
Seasons						
Summer	4.80± 0.048[d]	5.74±0.084[b c]	63.78±1.22[b]	33.05±0.30[b]	11.46±0.23	11.04±0.21[c]
Autumn	5.25±0.062[c]	5.63±0.109[c]	71.42±1.58[a]	34.25±0.38	12.07±0.30	10.86±0.27[d]
Winter	6.75±0.054[a]	5.99±0.095[a b]	62.81±1.38[c]	35.49±0.33	11.68±0.26	12.94±0.24[b]
Spring	6.12±0.087[b]	6.26±0.152[a]	62.01±2.21[d]	34.66.54	11.79±0.43	16.26±0.38[a]
Age group						
Pre-pubertal	5.68±0.041	5.86±0.071	62.99±1.03	33.03±0.25	11.45±0.20	12.58±0.18
Post pubertal	5.78±0.048	5.95±0.083	67.02±1.21	35.69±0.29	12.05±0.23	12.97±0.21

Table 3: Mean ± SE of sodium concentration, potassium concentration, chloride concentration and Calcium concentration

Particulars	Sodium (mEq/L)	Potassium (mEq/L)	chloride (mg/dl)	Calcium (mg/dl)
Energy levels				
Low energy level	146.18±0.274	5.08±0.036[b]	361.47±0.95[a]	107.96±1.25[b]
Medium energy level	146.13±0.279	5.20±0.037[a]	355.30±0.97[b]	103.50±1.27[c]
High energy level	146.28±0.284	5.18±0.037[a]	357.44±0.99[b]	111.97±1.30[a]
Seasons				
Summer	144.63±0.257[c]	4.84±0.034[c]	355.2±0.89[b]	108.40±1.17[b]
Autumn	146.60±0.332[b]	4.93±0.044[c]	357.30±1.154[b]	104.30±1.52[c]
Winter	145.84±0.289[b]	5.29±0.038[b]	357.30±1.004[b]	106.90±1.32[b]
Spring	147.72±0.464[a]	5.54±0.061[a]	362.60±1.61[c]	111.60±2.12[a]
Age group				
Pre-pubertal	143.66±0.217	5.04±0.028	356.95±0.75	106.03±0.99
Post pubertal	148.74±0.255	5.27±0.033	359.19±0.88	109.56±1.16

Chloride: The lowest values (355.3 ±0.97 mg/dl) for chloride contents were recorded in the group fed on medium energy levels, whereas, these values were highest in the group fed on low energy (361.47 ±0.95 mg/dl). These differences among the values were significant (P<0.01). The seasons of the year showed variations in chloride ion concentration. These variations were significantly different from each other during the experimental period (P<0.01). Highest values were recorded during spring i.e. 362.95mg /dl, while, lowest during summer. Chloride concentration was not affected by age. Arosh *et al*. (1998) observed that anoestrous cows had significant low concentration of chloride. They stated that the mineral plays an intermediate role in the action of hormones and enzymes at sub cellular levels. The mineral acts in integrated fashion in the synthesis of reproductive hormones, with positive action of such hormones on reproductive organs and initiation of estrus in animals.

Calcium: The different energy level in the rations exhibited the highly significant effects in serum calcium level. It was found that serum calcium level was increased in group of buffaloes fed on higher energy ration, Shemesh *et al*. (1984) stated that calcium dependent mechanism is involved in steroid biosynthesis in ovaries. The seasons of the year also affected the calcium level in blood. The lowest calcium level was recorded during autumn, while highest levels were obtained during spring season. These differences in values were significant (P<0.05). A significantly low level of calcium was noticed during pre-pubertal stage and increased after the attainment of puberty. Kumar *et al*. (1992) observed that average calcium level followed an increasing trend with basal level of in postnatal calves. It was high in the group of sexually mature heifers as compared to other developmental stages.

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