Effect of Feeding Ammoniated Wheat Straw Treated with Hydrochloric Acid on Blood Biochemical Profile in Growing Male Buffalo *(Bubalus bubalis)* Calves

P. K. Naik[1], Usha R. Mehra, Kalicharan[2], V. P. Varshney[3] and R. S. Dass*

Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar (U.P.)- 243 122, India

ABSTRACT : An experiment was conducted to study the effect of feeding ammoniated wheat straw treated with HCl on blood biochemical profiles in growing male buffalo *(Bubalus bubalis)* calves. Twenty-four growing male buffalo calves (one year of age, 88.54±3.81 kg average body weight) were divided into three groups in a completely randomized design on the basis of their body weight. Animals in all the three groups were fed on concentrate mixture. In addition, they were offered wheat straw, ammoniated wheat straw (4% urea at 50% moisture level) and HCl treated ammoniated wheat straw (4% urea at 50% moisture level and HCl added to trap 30% of the NH_3 evolved) in groups I, II and III, respectively for a period of 180 days, as per Kearl (1982) for body weight gain of 500 g/d. In all diets, concentrate:roughage ratio was fixed at 50:50 and were made isonitrogenous by adjusting CP levels of conc. mixtures. Blood was collected from jugular vein of each buffalo calf at the beginning and subsequently at two months interval of experimental feeding. Due to urea-ammoniation, the CP content of wheat straw increased from 2.90 to 6.96% and addition of HCl along with urea further increased the CP content to 10.09%. In all the three groups, the mean values of plasma glucose (mg %) and serum globulin (g %), showed a decreasing trend, while the mean value of serum TP (g %), serum A:G ratio, serum urea (mg %), serum creatinine (mg %), serum ALP (KA units), SGOT (units/ml.), SGPT (units/ml), serum T_3 and T_4 (ng/ml) showed an increasing trend with the advancement of feeding period. The cumulative period mean values of serum TP (6.15 to 6.20 g %), serum albumin (3.07 to 3.18, g %), serum globulin (2.98 to 3.09, g %), serum A:G ratio (1.03 to 1.10), serum ALP (23.15 to 23.63, KA units), serum T_3 (1.20 to 1.23 ng/ml) and serum T_4 (21.33 to 21.88 ng/ml) were comparable among the groups. The cumulative period mean plasma glucose (mg %) in group III (57.28) was similar to groups I (55.31) and II (59.41), however, the cumulative period mean plasma glucose in group II was significantly ($p<0.01$) higher than group I. The cumulative period mean serum urea (mg %) in group III (47.34) was significantly ($p<0.001$) higher than group I (38.38) and II (42.24), which were statistically alike. However, the cumulative period mean serum creatinine values (mg %) in groups II (1.43) and III (1.52) were similar and were significantly ($p<0.01$) higher than group I (1.24). The cumulative period mean SGOT (units/ml) in groups I, II and III was 91.71, 96.04 and 96.64, respectively. Similarly the cumulative period mean SGPT (units/ml) was 19.00, 19.93 and 20.01 in groups I, II and III, respectively. The cumulative period mean values of SGOT ($p<0.05$) and SGPT ($p<0.001$) in groups II and III were similar and were significantly higher than group I. The cumulative period mean serum T_3 and T_4 values in groups I (1.21 and 21.81), II (1.23 and 21.42) and III (1.20 and 21.33) were comparable. From the present study it may be concluded that feeding of AWS treated with and without HCl to growing male buffalo calves for 180 days had no significant adverse effect on blood biochemical profile. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 2: 185-191)***

Key Words : Ammoniated Wheat Straw, HCl, Buffalo Calves, Blood Urea, Serum Protein, Serum T_3, Serum T_4

INTRODUCTION

The main hindrance in utilisation of crop-residues are their low nutritive value and poor palatability. Out of the several methods tried in India and abroad to enhance the nutritive value of crop-residues, urea-ammoniation has been found to be the most promising, practicable and users friendly (Mehra et al., 1989; Khan et al., 1999; Fadal Elseed et al., 2003; Sarwar et al., 2003). But the losses of ammonia-nitrogen during urea-ammoniation of straw has been reported to be as high as 60-66% (Mandal et al., 1995; Dass et al., 2000). Various acids have been used to trap this excess ammonia (Borhami et al., 1982; Dass et al., 2001; Mehra et al., 2001) with different degree of success. But there is very little information on the effect of feeding ammoniated wheat straw treated with hydrochloric acid on the health status of the animals. Since the health status of the animals is reflected in the blood biochemical profile, an experiment was conducted to study the effect of feeding ammoniated wheat straw treated with hydrochloric acid on the blood biochemical profile of growing male buffalo *(Bubalus bubalis)* calves.

* Corresponding Author: R. S. Dass. Tel: +91-581-2301318, Fax: +91-581-247284, E-mail: rsd@ivri.up.nic.in

[1] P.A.U., Ludhiana 141004 (Punjab), India.

[2] Pathology Division, India.

[3] Physiology and Climatology Division, India.

Received May 21, 2003; Accepted June 25, 2004

MATERIALS AND METHODS

Preparation of concentrate mixture

To make the diet isonitrogenous three different types of concentrate mixtures namely CM_1, CM_2 and CM_3 (Table 1) were prepared. The CP content of CM_1, CM_2 and CM_3 was 22.07, 18.97 and 17.05%, respectively. Vitablend was added @ 25 g/100 kg concentrate mixture to meet out the vitamin A and D_3 requirement.

Table 1. Composition of concentrate mixtures

Ingredients	Parts by weight		
	CM_1	CM_2	CM_3
Maize	58	62	66
Soybean meal	39	28	19
Wheat bran	-	7	12
Mineral mixture	2	2	2
Common salt	1	1	1
Total	100	100	100
Calculated CP (%)	22.18	19.01	16.38
Calculated TDN(%)	77.40	77.41	77.58

Ammoniation of wheat straw

Ammoniated wheat straw without HCl (type A) and with HCl (type B) were prepared. In type 'A', wheat straw was treated with 4% fertilizer grade urea at 50% moisture level and in type 'B', wheat straw was treated simultaneously with 4% fertilizer grade urea and 3.5 litres hydrochloric acid (specific gravity 1.18 and purity 35%) to trap 30% of the free ammonia evolved. In both the cases, treated wheat straw was covered with polythene sheet and was kept air tight at room temperature for 21 days, as described by Dass et al. (1984).

Animals management and feeding

Twenty four growing male buffalo *(Bubalus bubalis)* calves (one year age, 88.54±3.81 kg average body weight) were randomly divided into three groups on the basis of their body weight. During the experiment, the animals were kept in well ventilated shed with individual feeding and watering arrangements. The animals were offered concentrate mixtures (CM_1 CM_2 and CM_3) along with wheat straw, ammoniated wheat straw and HCl treated ammoniated wheat straw in groups I, II and III, respectively for a period of 180 days as per Kearl (1982) to meet the nutrients requirement for 500 g gain/day. In all diets, the concentrate: roughage ratio was fixed at 50:50 and were made isonitrogenous. Roughage part was fed *ad libitum* to the experimental animals only after complete consumption of respective concentrate mixtures. Clean and fresh drinking water was provided *ad libitum* to all the experimental animals twice daily at 10 am and 3.30 pm.

Collection and processing of blood samples

Before feeding and watering of the experimental animals, about 10 ml, blood was collected from the jugular vein of each buffalo calf at the beginning and subsequently at two months interval of the experimental feeding.

Out of the 10 ml collected blood, 6 ml was taken in clean, dry test tube and was centrifuged at 3,000 rpm for 10 min for separation of serum. The remaining 4 ml blood was pipette into clean dry test tube containing a pinch of anticoagulant (mixtures of EDTA and Na_2F) and was shaken gently for proper mixing of whole blood with the anticoagulant and was centrifuged at 3,000 rpm for 10 min for separation of plasma. The respective serum and plasma samples were collected in plastic vials and were preserved in deep freezer (-20°C) till the completion of biochemical analysis. During the entire period of collection and processing, precautions were taken to avoid haemolysis of the blood samples.

Analytical techniques

Plasma glucose was estimated by the glucose oxidase (GOD) and peroxidase (POD) method, as described by Henry (1963). Serum total protein and albumin were determined by the modified Biuret and Dumas method as described by Dumas et al. (1971). Serum globulin was calculated by subtracting serum albumin from serum total protein. The serum A:G ratio was calculated by dividing the values of serum albumin by serum globulin. Diacetyl mono oxime (DAM) method as described by Wybenga et al. (1971) was followed to estimate the concentration of serum urea. The concentration of creatinine in serum was determined by alkaline picrate method as described by Bonses and Tausskay (1945). The serum alkaline phosphatase activity was estimated as per the method of Kind and King (1954). SGOT and SGPT were determined by the method suggested by Reitman and Frankel (1957). The concentrations of T_3 and T_4 were estimated by following the method of Bhandarkar and Pillai (1982).

Statistical analysis

Data were subjected to the statistical analysis by following the standard procedures (Snedecor and Cochran, 1980) by statistical software package (SPSS) for the test of significance.

Table 2. Chemical composition (% DM basis) of feeds

Attributes	Concentrate mixture			Roughage		
	CM_1	CM_2	CM_3	WS	AWS	HCI-AWS
Organic matter	92.05	92.12	92.76	92.55	91.81	91.92
Crude protein	22.0.7	18.97	17.05	2.90	6.96	10.09
Ether extract	1.52	1.54	1.51	1.84	1.68	1.53
Neutral detergent fiber	37.13	39.77	38.95	85.89	86.26	83.42
Acid detergent fiber	6.63	7.25	7.18	55.55	63.39	64.15
Cellulose	6.01	6.17	5.99	49.61	55.09	55.48
Hemi-cellulose	30.50	32.52	31.77	30.34	22.87	19.27

WS: wheat straw; AWS: Ammoniated wheat straw; HCl-AWS: HCl treated ammoniated wheat straw.

Table 3. Blood biochemical profile in buffalo calves

Group	Period (months post feeding)					Period	SEM		Interaction
	0	2	4	6	SEM	mean	Group	Period	
Plasma glucose (mg %)				**		**			NS
I***	62.23^{a}	61.09^{a}	52.80^{b}	45.13^{cy}	1.26	55.31^{y}	0.83	0.95	
II***	63.09^{a}	62.87^{a}	58.09^{b}	53.58^{bx}	1.60	59.41^{x}			
III***	63.50^{a}	62.79^{a}	54.45^{b}	48.39^{cy}	2.01	57.28^{xy}			
SEM	1.64	1.55	1.76	1.66					
Group mean ***	62.94^{a}	62.25^{a}	55.11^{b}	49.04^{c}					
Serum TP (g %)									NS
I	6.05	6.13	6.19	6.28	0.17	6.16	0.08	0.10	
II	6.04	6.12	6.19	6.25	0.13	6.15			
III	6.05	6.14	6.25	6.33	0.19	6.20			
SEM	0.17	0.19	0.14	0.17					
Group mean	6.05	6.13	6.21	6.29					
Serum albumin (g %)									NS
I***	2.80^{b}	2.96^{b}	3.26^{a}	3.28^{a}	0.06	3.07	0.04	0.04	
II***	2.84^{b}	2.95^{b}	3.40^{a}	3.50^{a}	0.08	3.18			
III***	2.85^{b}	2.86^{b}	3.39^{a}	3.56^{a}	0.08	3.16			
SEM	0.07	0.06	0.08	0.09					
Group mean***	2.83^{b}	2.92^{b}	3.35^{a}	3.45^{a}					
Serum globulin (g %)									NS
I	3.24	3.17	2.93	3.00	0.18	3.09	0.08	0.10	
II*	3.20^{a}	3.17^{a}	2.79^{b}	2.75^{b}	0.13	2.98			
III	3.20	3.29	2.87	2.65	0.18	3.03			
SEM	0.17	0.18	0.13	0.18					
Group mean**	3.21^{a}	3.21^{a}	2.86^{b}	2.84^{b}					
Serum A:G ratio									NS
I	0.90	0.97	1.13	1.12	0.08	1.03	0.04	0.04	
II***	0.90^{b}	0.94^{b}	1.23^{a}	1.32^{a}	0.07	1.10			
III****	0.92^{b}	0.90^{b}	1.21^{a}	1.32^{a}	0.07	1.08			
SEM	0.06	0.06	0.06	0.09					
Group mean***	0.90^{b}	0.93^{b}	1.19^{a}	1.25^{a}					
Serum urea (mg %)			*	*		***			NS
I***	26.88^{d}	34.53^{c}	41.93^{by}	50.19^{ay}	1.59	38.38^{y}	1.45	1.68	
II***	26.78^{c}	37.22^{b}	43.54^{by}	61.40^{axy}	3.29	42.24^{y}			
III***	26.89^{c}	39.50^{b}	56.50^{ax}	66.45^{ax}	3.45	37.34^{x}			
SEM	0.91	1.68	3.39	4.31					
Group mean***	26.85^{d}	37.09^{c}	47.32^{b}	59.35^{a}					
Serum creatinine (mg %)			*			***			NS
I***	0.79^{c}	0.80^{c}	1.61^{by}	1.77^{a}	0.05	1.24^{y}	0.04	0.05	
II***	0.80^{b}	0.95^{b}	1.92^{ax}	2.07^{a}	0.09	1.43^{x}			
III***	0.80^{c}	1.13^{b}	1.97^{ax}	2.19^{a}	0.10	1.52^{x}			
SEM	0.04	0.09	0.09	0.09					
Group mean***	0.80^{d}	0.96^{c}	1.83^{b}	2.01^{a}					

NS: non significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

$^{a, b, c, d}$ Means with different superscripts in a row differ significantly.

$^{x, y, z}$ Means with different superscripts in a column differ significantly.

RESULTS AND DISCUSSION

The physical and chemical composition of the various feeds are presented in Tables 1 and 2, respectively. Due to urea-ammoniation, the CP content of wheat straw increased from 2.90 to 6.96%. This might be due to the binding of ammonia released from the hydrolysis of urea inside the intermolecular spaces of wheat straw (Dass et al., 1984; Reddy et al., 1989). However, addition of HCl along with urea during urea-ammoniation further increased the CP content to 10.09%. This might be due to the trapping of the excess ammonia by forming ammonium chloride (Dass et al., 2001; Nair et al., 2002). It was evident from Table 2 that there was some increase in cellulose content in urea-ammoniated wheat straw, which further increased due to combined effect of HCl and urea during urea-ammoniation. The hemicellulose and soluble residues contents decreased due to urea ammoniation and addition of HCl during urea ammoniation resulted in further reduction.

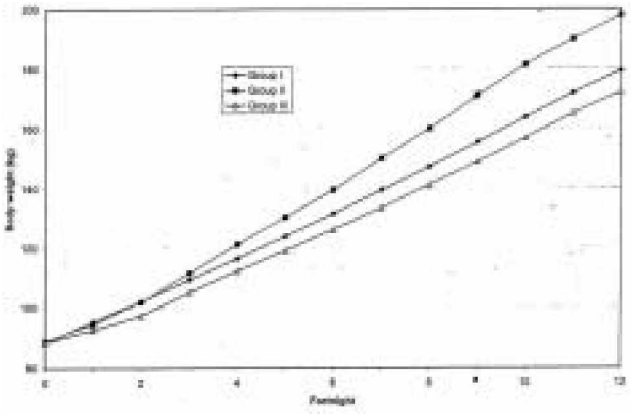

Figure 1. Fortnightly BW change in buffalo calves.

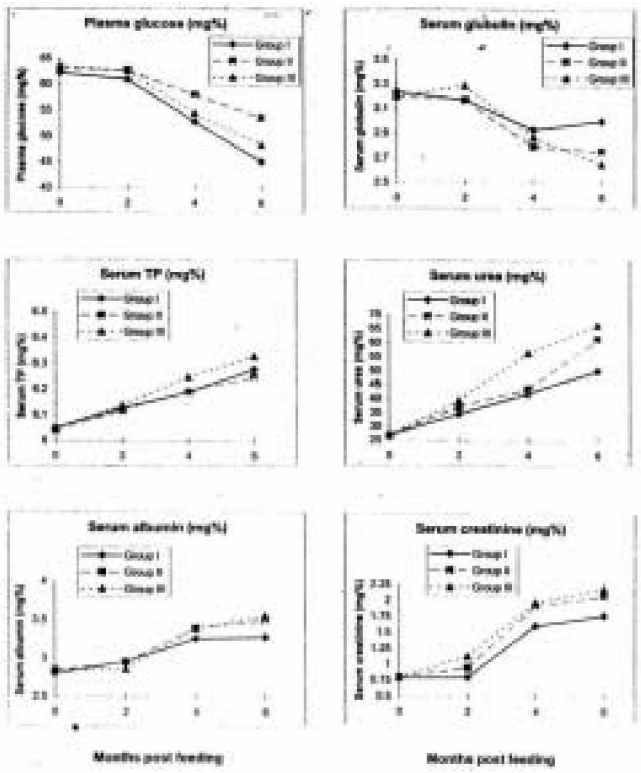

Figure 2. Effect of feeding ammoniated and ammoniated plus hydrochloric acid treated wheat straw on blood biochemical constituents.

At the start of the experiment, the mean body weight (kg) of buffalo calves in groups I, II and III was 88.3, 88.9 and 88.5, respectively and were comparable among the groups. At the end of the experimental period of 180 days, the mean body weight (kg) was 179.1, 197.8 and 172.0 in groups I, II and III, respectively and were also comparable among the groups. The gain in body weight (kg) during the period of 180 days was 90.9, 109.0 and 83.5 in groups I, II and III, respectively. The corresponding values for ADG (g) was 504.86, 605.56 and 463.89 in groups I, II and III, respectively. The mean values of BW gain (kg) and ADG (g) in group III were significantly ($p<0.05$) lower than group II and the mean values in both groups were similar to group I. The fortnightly body weight changes in buffalo calves in all three groups are presented in Figure 1. In all the three groups, there was an increase in BW with the advancement of feeding period and age.

The results of the blood biochemical profile in buffalo calves are presented in Table 3 and depicted in Figure 2. There was a lowering trend in the level of plasma glucose with advancement of age. These observations are depiction of higher plasma glucose in young animals which is tending to achieve its normal adult stage level. Similar period wise decreasing trend was also reported by earlier workers in buffalo calves (Puri et al., 1983; Helal et al., 1999) and crossbred calves (Sikka et al., 1994; Pattanaik, 1997). In group II, the values at 6 months post feeding (53.58 mg %) and the cumulative period mean value (59.41 mg %) remained significantly ($p<0.01$) higher than group I (45.13 and 55.13 mg %). This might be due to the higher OM intake in group II than group I. (96.45 g/kg $W^{0.75}$ vs. 89.99 g/kg $W^{0.75}$). However, the similar cumulative period mean plasma glucose in group III (57.28 mg %) with group II (59.41 mg %) ascribed no impact of addition of HCl during ammoniatoin. The mean serum TP values observed in this experiment are higher than values reported by Sil (1992) and slightly lower than values (7.75 g %) reported by Singh (1991) in buffalo calves fed on AWS based ration. However, the values obtained in this experiment were with in normal range of 5.7 to 8.1 g/dl and were comparable to those values observed by Nawaz and Siddiqui (1974). The gradual increasing trend in value of serum TP in all three groups might be due to the effect on their growth. This is also supported by the findings of Afzal and Anjum (1982) in buffalo calves. The comparable serum TP at 0, 2, 4 and 6 months post feeding and the comparable cumulative period mean values (6.16, 6.15 and 86.20 g %) among the groups revealed that the protein available from AWS and HCl-AWS for absorption at intestinal level were similar to control diet. Similarly, no effect of sources of proteins on serum TP was recorded by earlier workers (Giri and Dass, 1993; Tiwari et al., 2001). The mean values of serum albumin were parallel with earlier findings (Verma et al., 1975). The significant ($p<0.001$) gradual increase in serum albumin values in all three groups might be due to their body weight gain. The mean values of serum globulin are similar to the values reported earlier (Sahoo, 1994; Pattanaik, 1997). The significant decreasing trend of serum globulin level in group II at 4 months post feeding and onwards might be due to the cyto-toxic effect of urea in AWS on lymphoid organs. However, the cumulative period mean serum globulin values (3.09, 2.98 and 3.03 g %) among the groups were with in normal range. The significant ($p<0.001$) increasing trend of serum A:G ratio at

Table 4. Blood enzyme and hormone concentration in buffalo calves

Group	Period (months post feeding)					Period	SEM		Interaction
	0	2	4	6	SEM	mean	Group	Period	
Serum ALP (KA units)									NS
I	19.73	22.22	24.77	25.86	2.23	23.15	1.13	1.30	
II	19.72	22.51	25.31	27.00	2.25	23.63			
III	19.36	22.98	25.30	26.83	2.26	23.62			
SEM	2.33	2.13	2.19	2.34					
Group mean **	19.60^{c}	22.57^{bc}	25.13^{ab}	26.56^{a}					
SGOP (units/ml)						*			NS
I	86.03	89.96	95.86	94.99	3.16	91.71^{y}	1.50	1.73	
II*	88.41^{b}	92.85^{ab}	101.30^{a}	101.61^{a}	3.46	96.04^{x}			
III***	89.90^{b}	93.72^{b}	100.74^{a}	102.23^{a}	2.21	96.64^{x}			
SEM	2.94	3.04	3.07	2.91					
Group mean***	88.11^{b}	92.18^{b}	99.30^{a}	99.61^{a}					
SGPT (units/ml)				***	***		***		NS
I**	18.05^{c}	18.57^{bc}	19.45^{aby}	19.93^{ay}	0.378	19.00^{y}	0.21	0.24	
II***	18.01^{b}	18.57^{b}	21.23^{ax}	21.91^{ax}	0.360	19.93^{x}			
III***	17.93^{b}	18.51^{b}	21.55^{ax}	22.04^{ax}	0.492	20.01^{x}			
SEM	0.46	0.46	0.38	0.34					
Group mean***	18.00^{b}	18.55^{b}	20.74^{a}	21.29^{a}					
Serum T_3 (ng/ml)						*			NS
I**	0.98^{b}	1.17^{b}	1.17^{b}	1.53^{ay}	0.11	1.21	0.05	0.05	
II***	0.93^{b}	1.10^{b}	1.10^{b}	1.79^{ax}	0.11	1.23			
III***	0.98^{c}	1.15^{b}	1.15^{b}	1.52^{ay}	0.05	1.20			
SEM	0.09	0.10	0.10	0.07					
Group mean***	0.96^{c}	1.14^{b}	1.14^{b}	1.61^{a}					
Serum T_4 (ng/ml)									NS
I***	18.55^{c}	21.08^{b}	22.93^{ab}	24.68^{a}	0.76	21.81	0.35	0.40	
II***	18.88^{c}	20.70^{bc}	21.97^{b}	24.14^{a}	0.71	21.42			
III***	18.48^{b}	19.71^{b}	22.86^{a}	24.27^{a}	0.61	21.33			
SEM	0.67	0.56	0.74	0.79					
Group mean***	18.64^{d}	20.50^{c}	22.59^{b}	24.36^{a}					

NS: non significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

$^{a, b, c, d}$ Means with different superscripts in a row differ significantly.

$^{x, y, z}$ Means with different superscripts in a column differ significantly.

4 months post feeding and onwards in group II could be attributed to the increasing and decreasing trend of serum albumin and globulin, respectively, and may be due to the cyto-toxic effect of urea of AWS on lymphoid organs. But the simultaneous addition of HCl during urea ammoniation of WS did not show any significant effect on the cyto-toxic property of AWS, as has been reflected by significant ($p<0.001$) increase in A:G ratio at 4 months post feeding and onwards in group III. However, the comparable cumulative period mean A:G ratio value among the groups were with in normal range and are supported by the findings of Ahuja et al. (1977). There was a significant ($p<0.001$) rising trend in mean serum urea values in all three groups. Similar trend was also noticed by Giri and Dass (1993) in buffalo calves. The serum urea level at 4 months post feeding and onwards was significantly ($p<0.05$) higher in group III than other two groups. The cumulative period mean serum urea (mg %) in group III was significantly ($p<0.01$) more than group I. This upswing, may presumably be either due to the more amount of NPN of the HCl treated AWS or the changes in vital organs like liver and kidney. Similarly, on supplementation of NPN compounds to the basal diet, high level of BUN was reported by earlier workers (Nolan and Leng, 1972; Dass et al., 1996; Bakshi et al., 1997).

Values of blood enzymes and hormones like T_3 and T_4 are given in Table 4 and depicted in Figure 3. The gradual increasing trend in mean values of serum ALP with advancement of age might be due to the normal physiological effect of their growth in all three groups. The cumulative period mean serum ALP values were comparable among three groups and were within normal range. Ahuja et al. (1977) also did not find any change in serum ALP concentration in buffalo bulls fed on urea for a prolonged period. The serum level of SGOT and SGPT were well with in normal range. But when the values were compared on the basis of the effect of ammoniation, then the mean values of SGOT in groups II ($p<0.05$) and III ($p<0.01$) and the mean values of SGPT in groups I and II ($p<0.001$) showed a significant increasing trend with

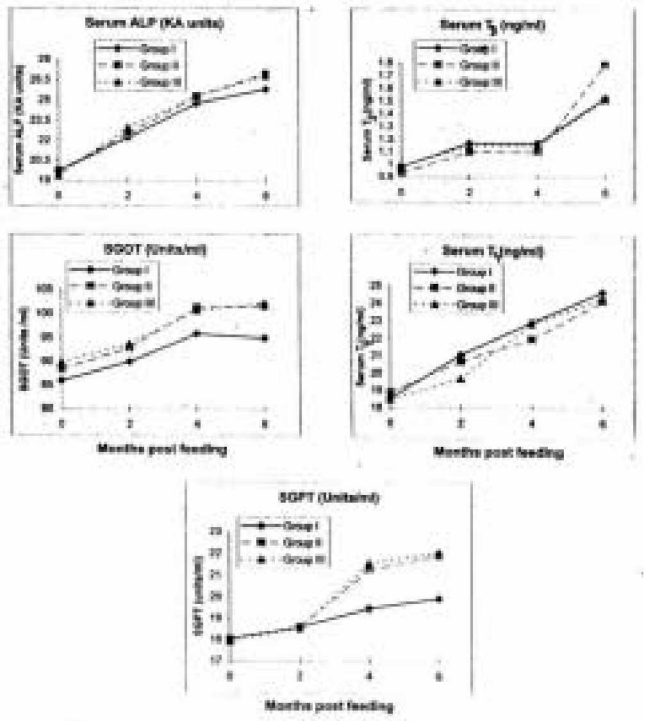

Figure 3. Effect of feeding ammoniated and hydrochloric acid treated wheat straw on some blood enzymes, T_3 and T_4 concentration in buffalo calves.

advancement of feeding. Similarly, the cumulative period mean values of SGOT ($p<0.05$) and SGPT ($p<0.001$) were significantly higher in groups II and III than group I. These higher values might be due to some pathological alterations in vital organs. The serum creatinine level at 4 months post feeding ($p<0.05$) and the cumulative period mean serum creatinine (mg %) values ($p<0.001$) in groups II (1.43) and III (1.52) were comparable and were significantly higher than group I (1.24). The changes might be due to the renal and muscular damage. However, the values found in the present study were well within normal range of 1.0 to 2.7 mg/dl. reported for the domestic animals (Kaneko, 1980). The mean values of serum T_3 and T_4 were well with in the normal physiological range. There was an increasing trend in both serum T_3 and T_4 values in all three groups. The cumulative period mean values of serum T_3 (1.21, 1.23 and 1.20 ng/ml) and T_4 (21.81, 21.42 and 21.33 ng/ml) in groups I, II and III were similar in all three groups, indicating no adverse effect of AWS and HCl-AWS on basal metabolic rate of buffalo calves.

CONCLUSION

From the study, it may be concluded that feeding of ammoniated wheat straw treated with and without HCl to growing male buffalo calves for 180 days had no significant adverse effect on any blood biochemical constituents.

REFERENCES

Afzal, M. and A. D. Anjum. 1982. Serum protein changes in buffalo calves after colostrum feeding. Pak. Vet. J. 2(4):182-183.

Ahuja, S. P., R. Saini and I. S. Bhatia. 1977. Effect of prolonged feeding of urea to buffalo bulls *(bubalus bubalis)* on their hepatic functions. Zentralblatt-fur-veterinarmedizin. 24A:320-323.

Bakshi, M. P. S., Y. M. Wadhawa, M. Wadhawa and P. N. Langar. 1997. Effect of supplementing processed poultry litter with urea treated straw preparations on the biochemical changes in rumen and blood parameters of buffaloes. Buffalo J. 13(2):127-134.

Bhandarkar, S. D. and M. I. A. Pillai. 1982. Radio-immunoassay: A laboratory manual-Bhabha Atomic Research Centre, Bombay, India.

Bonses, R. W. and H. H. Tausskay. 1945. The colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem. 158:581.

Borhami, B. E. A, F. Sundstol and T. H. Garmo. 1982. Studies on ammonia treated straw: 2. Fixation of ammonia in treated straw by spraying with acids. Anim. Feed. Sci. Technol. 7(1):53-59.

Colin-negrete, J., H. E. Kiesling, T. T. Ross and J. F. Smith. 1996. Effect of whole cottonseed on serum constituents, fragility of erythrocyte cells and reproduction of growing Holstein heifers. J. Dairy Sci. 79:2016-2023.

Dass, R. S., A. K. Verna and U. R Mehra. 1996. Effect of feeding urea molasses liquid diet on nutrient utilization, rumen fermentation pattern and blood profile in adult male buffaloes. Buffalo J. 12(1):11-22.

Dass, R. S., A. K. Verma, U. R. Mehra and D. S. Sahu. 2001. Nutrient utilization and rumen fermentation pattern in Murrah buffaloes *(Bubalus bubalis)* fed urea and urea plus hydrochloric acid treated wheat straw. Asian-Aust. J. Anim. Sci. 14(2):1542-1548.

Dass, R. S., J. Kishan and U. B. Singh. 1984. Effect of feeding urea (ammonia) treated paddy straw on rumen metabolism in crossbred cattle. Indian J. Nutr. Dietet. 21(9):342-349.

Dass, R. S., U. R. Mehra and A. K. Verma. 2000. Nitrogen fixation and *in situ* dry matter and fiber constituents disappearance of wheat straw treated with urea and boric acid in Murrah buffaloes. Asian-Aust. J. Anim. Sci. 11:1133-1136.

Dumas, B. T., W. A. Watson and H. G. Briggs. 1971. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta. 31:87-96.

Fadal Elseed, A. M. A., J. Sekins, M. Hishinuna and K. Hamana. 2003. Effects of ammonia, urea plus calcium hydroxide and animal urine treatments on chemical composition and *in sacco* degradability of rice straw. Asian-Aust. J. Anim. Sci. 16:368-373.

Giri, S. S. and R. S. Dass. 1993. Effect of feeding formaldehyde treated mustard cake on blood metabolites in buffalo calves fed on ammoniated straw. Indian J. Dairy Sci. 46(11):505-509.

Helal, F. I. S., E. I. M. A. Ashry, H. S. Soliman and H. M. Aly. 1999. Newly born buffalo calves receiving milk replacers containing different sources and levels of energy density (2).

Incorporating high levels of homogenized margarnine in local milk replacers for rearing young buffalo calves. In Proc. VII scientific conf. on Anim. Nutr. (2):459-471.

Henry, R. J. 1963. Standard methods of clinical chemistry. Ist edn., Harper and Row Publishing Company, New York, USA.

Kaneko, J. J. 1980. Clinical Biochemistry of Domestic Animals (3rd edn.) Academic press Inc. orlands, Florida 32887.

Kearl, L. C. 1982. Nutrient Requirements of Ruminants in Developing Countries. International feed stuffs institute, Agriculture Experiment station, Utah State University, Logan, Utah, pp. 109-112.

Khan, M. J., J. R. Scaife and F. D. Hovell. 1999. The effect of different sources of urease enzyme on the nutritive value of wheat straw treated with urea as a source of ammonia. Asian-Aust. J. Anim. Sci. 12 (7):1063-1069.

Kind, P. R. M. and E. J. King. 1954. Estimation of serum alkaline phosphatase activity by colorimetric method. J. Clinical Path. 7:322.

Mandal, A., B. Sil, A. Santra, D. N. Kamra and N. N. Pathak. 1997. An alternate technique of ammoniation by ensiling straw with urea to minimize loss of added nitrogen. J. Appl. Anim. Res. 11(2):195-200.

Mehra, U. R., N. N. Pathak, U. B. Singh and R. S. Dass. 1989. Studies on the nutritional improvement of sorghum stover (*Jowar Kadbi*) through ammoniation by urea ensiling. Biol. Wastes 29(3):67-71.

Mehra, U. R., R. S. Dass, A. K. Verma and D. S. Sahu. 2001. Effect of feeding urea and acetic acid treated wheat straw on the digestibility of nutrients in adult male Murrah buffaloes *(Bubalus bubalis)*. Asian-Aust. J. Anim. Sci. 14(12):1690-95.

Nair, V. P., A. K. Verma, R. S. Dass and U. R. Mehra. 2002. Growth and nutrient utilization in buffalo calves fed urea-ammoniated wheat straw and hydrochloric acid plus urea treated wheat straw. Asian-Aust J. Anim. Sci. 15(5):682-686.

Nawaz, M. and R. H. Siddiqui. 1974. Normal pH of blood, total serum proteins and cholesterol in the serum of female buffaloes Nordisk-veterinaermedicin 26(3-4):211-214.

Nolan, J. V. and R. A. Leng. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. Br. J. Nutr. 27:117.

Pattanaik, A. K. 1997. Effect of synchronization of degradable protein and starch on the performance of crossbred calves. Ph.D. Thesis, Deemed Univ., IVRI, Izatnagar, India.

Puri, J. P., O. P. Nangia, S. L. Garg, J. S. Punia and Narendra Singh. 1983. Early development of rumen function in buffalo calves, nitrogen metabolism and blood metabolites as influenced by age and diet. Indian J. Anim. Sci. 53 (8):880-883.

Reddy, D. V., U. R. Mehra and U. B. Singh. 1989. Effect of hydrogen peroxide treatment on the utilization of lignocellulosic residues by rumen micro-organisms. Biol. Wastes. 28(2):133-141.

Reitman, S. and S. Frankel. 1957. A colorimetric method for the determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transminase. Am. J. Clin. Pathol. 28:56-63.

Sahoo, A. 1994. Nutritional studies on the performance of faunated and defaunated crossbred calves during pre-weaning and post-weaning period, Ph.D. Thesis, Deemed Univ., IVRI, Izatnagar, India.

Sarwar, M., M. Ajmal Khan and Mahr-Un-Nisa. 2003. Nitrogen retention and chemical composition of urea treated wheat straw ensiled with organic acids or fermentable carbohydrates. Asian-Aust. J. Anim. Sci. 16(11):1583-1592.

Sikka, P., R. K. Sethi, A. K. S. Tomar and S. C. Chopra. 1994. Blood metabolites level in relation to age and live weight in young buffalo calves. Asian-Aust. J. Anim. Sci. 7(2):201-205.

Sil, B. 1992. Comparative studies on feeding of different conventional proteins in urea containing diets on performance of crossbred calves. M.V.Sc., Thesis, Deemed Univ., IVRI, Izatnagar.

Singh, P. 1991. Utilization of nutrient from straw as affected by mode of NPN supplementation. Ph.D. Thesis, Deemed Univ., IVRI, Izatnagar, India.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical Methods, 7th edn. The Iowa state Univ. Press, Ames, Iowa, USA.

Tiwari, C. M., A. S. Chandramoni, S. B. Jadhao, S. K. Gowda and M. Y. Khan. 2001. Studies on blood biochemical constituents and rumen fermentation in growing buffalo calves fed ammoniated straw based rations, supplemented with different protein sources. Anim. Feed Sci. Technol. 89:119-130.

Verma, D. N., C. B. Johri and S. K. Ranjhan. 1975. A note on certain blood biochemical constituents of buffalo calves fed on different levels of urea in the diet. Indian J. Anim. Sci. 45(8):580-582.

Wybenga, Donald R., John Digiorgio and Vincant J. Pileggi. 1971. Manual and automated methods for urea nitrogen measurement in whole serum. Clin. Chem. 17:891.