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Original article

Effect of long term feeding of ammoniated wheat straw treated with or without HCl on blood biochemical parameters in growing male buffalo (*Bubalus bubalis*) calves

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Abstract – Twenty-four growing male buffalo calves (one year of age; 88.54 ± 3.81 kg average body weight) were divided into three comparable groups (I, II and III) on the basis of their body weight (BW) in a completely randomised design to study the effect of long term feeding of ammoniated wheat straw (AWS) and hydrochloric acid treated ammoniated wheat straw (HCl-AWS) on blood biochemical changes. The animals were offered a concentrate mixture (CM) along with wheat straw (WS), ammoniated wheat straw (AWS) (4% urea at a 50% moisture level) and hydrochloric acid treated ammoniated wheat straw (HCl-AWS) (4% urea at a 50% moisture level and HCl added to trap 30% of NH_3 evolved) in groups I, II and III, respectively for an average daily gain (ADG) of 500 g. All the diets were made iso-nitrogenous by preparing three types of concentrate mixtures of different CP levels. The blood was collected from the jugular vein randomly from three animals of each group initially after 8 months post feeding and subsequently after two months interval up to 14 months of experimental feeding. Due to urea ammoniation, the CP content of WS increased from 3.66 to 8.51 and was further increased to 11.35 due to the addition of HCl during urea-ammoniation of wheat straw. The cumulative period mean plasma glucose values (mg %), in group II (53.13) were significantly ($P < 0.001$) higher than those in groups I (48.44) and III (50.60). The cumulative period mean values of serum albumin and globulin (g %) were not significantly different and were comparable among the groups I (3.33 and 3.06), II (3.53 and 2.97) and III (3.49 and 2.94). The cumulative period mean values of serum albumin: globulin ratio and total protein values were not significantly different among the different groups. Serum urea and creatinine values were significantly ($P < 0.001$) higher in group III (58.66 and 2.24) as compared to groups I and II. The cumulative period mean values of serum alkaline phosphatase (ALP) (KA units) did not differ significantly, but serum glutamate pyruvate transaminase (SGPT) and glutamate oxaloacetate transaminase (SGOT) values (units·mL^{-1}) were significantly ($P < 0.001$) higher in groups II and III than in group I. The cumulative period mean values of T_3 (ng·mL^{-1}) did not differ significantly among the groups, but T_4 values were significantly ($P < 0.001$) higher in group III (22.74) than in groups I (21.41) and II (20.89), respectively. Since the mean values of all the blood parameters were within the normal range, it may be concluded that feeding of ammoniated wheat straw treated with and without HCl to growing male buffalo calves for fourteen months has no adverse effect on the blood biochemical parameters.

ammoniated wheat straw / buffalo calves / blood biochemical / HCl-AWS / T_3 / T_4

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1. INTRODUCTION

Livestock feeding in India and other developing countries depends mainly upon the poor quality crop residues. However, because of an excessive lignification, lower digestibility and poor palatability, animal performance is restricted [1]. Various chemicals have been tried by earlier workers [2–5] to break the lignocellulosic complex in order to improve the nutritive value of these crop residues. Fertiliser grade urea-ammoniation of crop residues has been found to be cheap and feasible method for nutritional improvement of poor quality roughages [6]. During urea ammoniation about 60–66% of the free ammonia released from urea goes to the atmosphere, leading to nutritional loss and environmental pollution [7–9]. In order to minimise the loss of ammonia during urea ammoniation, various organic and inorganic acids have been employed to trap the excess ammonia with different degrees of success [10–12]. Acid treated or untreated ammoniated straws have been fed successfully to cattle and buffaloes but there is very little information on the effect of long term feeding of ammoniated wheat straw on the health status of the animals. Therefore, the present experiment was conducted to study the effect of long term feeding of ammoniated wheat straw (AWS) treated with (HCl-AWS) or without HCl on the blood biochemical profile in growing male buffalo (*Bubalus bubalis)* calves, since the blood biochemical profile reflects the health status of the animals.

2. MATERIALS AND METHODS

2.1. Ammoniation of wheat straw with and without hydrochloric acid

Ammoniated wheat straw without HCl (AWS) and ammoniated wheat straw treated with HCl (HCl-AWS) were prepared separately. For ammoniation without HCl, wheat straw was treated with 4% fertiliser grade urea at a 50% moisture level. However, for ammoniation with HCl, wheat straw was treated simultaneously with 4% fertiliser grade urea and 3.5 L HCl (specific gravity 1.18 and purity 35%) to trap 30% of the free ammonia evolved during urea ammoniation. In both cases, treated wheat straw was covered with a polythene sheet and kept air tight at room temperature for 21 days as described earlier [13].

2.2. Management and feeding of the animals

The study was conducted on twenty-four growing male buffalo (*Bubalus bubalis*) calves (one year of age; 88.54 ± 3.87 kg average body weight), divided randomly into three groups (I, II and III) on the basis of their live body weight (BW). All the animals were kept in well ventilated sheds with individual feeding and watering arrangements and 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 in order to meet their nutrient requirement for a daily gain of 500 g·d^{-1} for a period of fourteen months as per Kearl [14]. To adjust the increased nitrogen intake through AWS and HCl-AWS and to make the diets isonitrogenous, three different types of concentrate mixtures (CM) namely CM_1, CM_2 and CM_3 (Tab. I) were prepared. The CP content of CM_1, CM_2 and CM_3 were 22.41, 18.14 and 14.16 percent, respectively. Vitablend was added at 25 g·100 kg^{-1} concentrate mixture to provide vitamins A and D_3 requirements. All the diets were iso-nitrogenous and the concentrate had a roughage ratio of 50:50. The roughage part was offered ad libitum to the experimental animals only after complete consumption of the respective concentrate mixture. Clean and fresh drinking water was provided ad libido to all the experimental animals twice daily at about 1000 and 1530 h.

2.3. Collection and processing of blood samples

Blood samples were collected randomly from three animals of each group at 0 d and

Table I. Physical composition of concentrate mixtures fed to animals of different groups*.

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

* Vitablend was added at 25 g·100 kg^{-1} concentrate mixture as a source of Vitamin A and D_3, 50 000 and 5 000 I.U.·g^{-1}, respectively.

Table II. Chemical composition (% DM basis) of untreated, ammoniated and HCl-treated ammoniated wheat straw.

Attributes	Roughage		
	WS	AWS	HCl-AWS
Organic matter	91.66	90.89	90.52
Crude protein	3.66	8.51	11.35
Ether extract	1.54	1.06	0.71
Neutral detergent fibre	86.68	85.32	83.45
Acid detergent fibre	55.62	63.85	61.66
Cellulose	44.10	49.81	48.65
Hemicellulose	31.06	21.47	21.79

subsequently at 2 months interval. Before feeding and watering, 10 mL blood was collected from the jugular vein for harvesting of serum and plasma, which was split into two samples. From the 10 mL sample, 6 mL was placed in a clean dry test tube and kept for an hour before it was centrifuged at 3000 rpm for 10 min for serum separation. The remaining 4 mL blood was pipetted into another clean and dry test tube containing an anticoagulant (mixtures of EDTA and Na_2F; 1 mg·mL^{-1} blood) and was shaken gently for proper mixing of whole blood with the anticoagulant and then centrifuged at 3000 rpm for 10 min. The respective serum and plasma samples, collected in plastic vials were preserved in a deep freezer (–20 °C) until the completion of biochemical analyses.

2.4. Analytical techniques

The WS, AWS, and HCl-AWS samples were analysed for organic matter, crude protein and ether extractives [15], neutral detergent fibre, acid detergent fibre, cellulose and hemi-cellulose [16]. Plasma glucose was determined by the glucose oxidase (GOD) and peroxidase (POD) method [17]. Serum total protein and albumin were also estimated [18]. Serum globulin was calculated by subtracting serum albumin from total serum protein. The serum A:G ratio was calculated by dividing serum albumin by serum globulin. Serum urea was determined by the diacetyl monoxime method [19]. The alkaline picrate method [20] was used to estimate serum creatinine concentration. Serum alkaline phosphatase activity was estimated as per the method of Kind and King [21]. Serum glutamate pyruvate transaminase (SGPT) and glutamate oxaloacetate transaminase (SGOT) were determined by the method suggested by Reitman and Frankel [22]. A radio immuno assay technique was used for the estimation of T_3 and T_4 in blood serum [23].

2.5. Statistical analysis

The data generated in this experiment were statistically analysed using repeated measures analysis for determining period effects and the interaction of period with group following a linear model as described by Snedecor and Cochran [24] and the treatment means were compared for significance [25].

3. RESULTS AND DISCUSSION

The physical composition of concentrate mixtures and chemical composition of roughages are presented in Tables I and II, respectively. Urea ammoniation without HCl increased the CP content of wheat straw

from 3.66 to 8.51% (42% of the total added urea nitrogen), which may be due to the binding of ammonia released from urea hydrolysis inside the intermolecular spaces of wheat straw [13]. However, urea-ammoniation with HCl further increased the CP content of wheat straw to 11.35% (25% of the total added urea nitrogen) as shown by chemical analysis of the straws given in Table II. This might be due to the trapping of the excess free ammonia by forming ammonium chloride [5, 12]. It was evident from Table II that there were some changes in the fibre fractions of the wheat straw due to urea ammoniation with and without HCl, which was also in agreement with the reports published earlier [5, 12].

The effect of feeding of ammoniated straw/HCl ammoniated straw on blood biochemical constituents is presented in Table III The results revealed a lowering trend in the values of plasma glucose with an increase of age. These observations reflect higher plasma glucose concentrations in young calves, which tends to decrease physiologically to reach normal values. Similarly, earlier workers observed a decreasing trend with an increase of age in buffalo calves [26, 27] and crossbred calves [28, 29]. The cumulative period mean plasma glucose (mg %) value in group II (53.13) was significantly ($P < 0.001$) higher than groups I (48.44) and III (50.60). However, the cumulative period mean plasma glucose values in groups I and III indicate that there was no impact of the addition of HCl during urea-ammoniation of wheat straw.

The serum protein level indicates the balance between anabolism and catabolism of protein in the body. The plasma protein concentration at any given time in turn is a function of hormonal balance, nutritional status, water balance and other factors affecting health. Total protein concentration in healthy animals normally varies between 6.0 and 7.9 g % and is altered during any liver and kidney diseases [30]. The cumulative period mean values of serum total protein (g %) and globulin (g %) in groups I (6.38 and 3.06), II (6.52 and 2.97) and III (6.43 and 2.94) were comparable and were not significantly different among the groups. The observations of earlier workers [31, 32] in cattle and buffalo calves respectively fed on ammoniated wheat straw, were similar. Serum albumin is synthesised by the liver, is catabolised by a wide variety of tissues and is an abundant plasma protein. Serum albumin supplies readily available pool of amino acids to meet tissue needs depending upon the nutritional status. Its synthesis is diminished during fasting, malnutrition, hormonal imbalances and poor condition of the liver and the serum globulins, mainly the α and β globulins are increased in acute inflammatory conditions such as acute hepatitis and glomerulonephritis and the γ globulins are mainly related with the immuno-status of the animal [33]. There was no effect of the period of feeding of ammoniated straw on the serum albumin level. The statistically similar, cumulative period mean serum albumin levels (g %), and serum A:G ratio values in groups I (3.33 and 1.14), II (3.53 and 1.24) and III (3.49 and 1.24) were well with in the normal range indicating no effect of long term feeding of AWS and HCl-AWS on the cyto-toxic property of lymphoid organs of the buffalo calves. The mean values of the serum A:G ratio observed were similar to the earlier observations in buffalo calves fed on urea for a prolonged period [34].

The serum urea concentration is closely associated with the break down and deamination of the protein in the rumen and the rate of utilisation of NH_3 for bacterial protein synthesis. An increase in the serum urea level may reflect an accelerated rate of protein catabolism rather than a decrease in urinary excretion [30]. The serum urea level also increases in renal tubular necrosis and decreases in hepatic insufficiency and low protein intake [35]. There was a significant ($P < 0.001$) difference in the cumulative period mean values of serum urea (mg %) among the groups, being the highest in group III (58.66) followed by group II (53.69) and the lowest in group I (47.00). This may be due

Table III. Bimonthly blood biochemical profile of buffalo calves fed urea ammoniated wheat straw treated with or without HCl in various groups.

Attributes	Group	Periods (month post feeding)								SEM	Mean	SEM		Interaction
		0	2	4	6	8	10	12	14			Group	Period	
	I	60.36	59.44	52.12	45.29	44.99	43.15	41.41	40.67	1.12	48.44^{x***}	3.73	1.36	NS
Plasma glucose (mg %)	II	62.59	62.51	56.68	52.27	52.06	49.06	44.77	45.09	1.33	53.13^{z}			
	III	64.55	63.79	55.10	49.26	46.91	42.96	42.00	40.23	1.84	50.60^{y}			
Mean***		62.50^{e}	61.95^{e}	54.63^{d}	48.94^{c}	47.99^{c}	45.06^{b}	42.73^{ab}	42.00^{a}		50.72			
SEM		1.83	1.74	2.39	2.08	0.37	0.47	0.50	0.47					
	I	6.30	6.41	6.21	6.31	6.28	6.27	6.23	6.83	0.16	6.38	0.18	0.13	NS
Serum protein (g %)	II	6.29	6.36	6.30	6.32	6.55	6.82	6.45	7.05	0.12	6.52			
	III	5.85	6.15	6.36	6.47	6.65	6.53	6.52	6.93	0.19	6.43			
Mean***		6.14^{a}	6.31^{ab}	6.29^{ab}	6.43^{b}	6.49^{b}	6.53^{b}	6.40^{b}	6.93^{c}		6.45			
SEM		0.17	0.16	0.16	0.20	0.16	0.15	0.14	0.08					
	I	3.52	3.47	3.00	3.23	3.15	2.68	2.48	2.91	0.17	3.06	0.21	0.14	NS
Serum globulin (g %)	II	3.42	3.33	2.82	2.84	3.07	3.01	2.39	3.02	0.17	2.97			
	III	3.03	3.34	2.97	2.98	3.11	2.76	2.53	2.82	0.21	2.94			
Mean***		3.32^{bc}	3.38^{c}	2.93^{b}	0.02^{bc}	3.11^{bc}	2.82^{b}	2.47^{a}	2.92^{b}		2.99			
SEM		0.18	0.22	0.22	0.10	0.16	0.18	0.21	0.19					
	I	2.78	2.94	3.20	3.28	3.19	3.60	3.75	3.92	0.12	3.33	0.21	0.09	NS
Serum albumin (g %)	II	2.87	3.03	3.49	3.47	3.49	3.81	4.06	4.03	0.11	3.53			
	III	2.81	2.80	3.39	3.49	3.54	3.79	3.98	4.11	0.11	3.49			
Mean***		2.82^{a}	2.93^{a}	3.36^{b}	3.42	3.38^{b}	3.73^{c}	3.93^{d}	4.02^{d}		3.45			
SEM		0.09	0.06	0.09	0.11	0.11	0.16	0.17	0.06					
	I	0.80	0.86	1.08	1.03	1.00	1.38	1.58	1.36	0.11	1.14	01.6	0.09	NS
Serum A:G ratio	II	0.84	0.92	1.25	1.25	1.15	1.30	1.83	1.35	0.13	1.24			
	III	0.96	0.85	1.18	1.21	1.18	1.44	1.61	1.46	0.12	1.24			
Mean***		0.87^{a}	0.87^{a}	1.17^{b}	1.16^{b}	1.11^{b}	1.37^{c}	1.68^{d}	1.39^{c}		1.20			
SEM		0.07	0.05	0.09	1.10	0.10	0.15	0.23	0.06					
	I	27.31	34.41	42.17	53.64	51.42	53.16	56.06	57.98	1.38	47.0^{x***}	6.89	2.92	NS
Serum urea (mg %)	II	25.80	35.44	41.57	65.93	62.43	65.44	66.78	66.13	2.63	53.69^{y}			
	III	25.78	38.08	61.55	67.45	65.88	67.81	71.42	71.34	3.59	58.66^{z}			
Mean***		26.29^{a}	35.97^{b}	48.43^{c}	62.34^{de}	59.91^{d}	62.14^{de}	64.75^{e}	65.15^{e}		53.12			
SEM		0.97	1.71	4.66	5.60	0.79	0.35	0.42	0.19					
	I	0.75	0.77	1.58	1.77	1.72	2.20	2.53	3.19	0.09	1.81^{y***}	0.90	0.12	NS
Serum creatinine (mg %)	II	0.80	0.94	2.03	2.23	2.25	2.68	2.94	3.55	0.14	2.18^{x}			
	III	0.80	0.97	1.92	2.17	2.68	2.88	2.94	3.57	0.11	2.24^{x}			
Mean***		0.79^{a}	0.89^{a}	1.84^{b}	2.06^{c}	2.22^{c}	2.59^{d}	2.80^{c}	3.43^{f}		2.08			
SEM		0.06	0.09	0.09	0.09	0.12	0.10	0.09	0.20					

NS: non significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ^{a,b,c,d,e} means with different superscripts in a row differ significantly.
^{x,y,z} Means with different superscripts in a column differ significantly.

to the higher amount of non-protein nitrogen of AWS and HCl-AWS, in groups II and III, respectively. There was a significantly ($P < 0.001$) increasing trend in mean serum urea values in all three groups. A similar trend was also noticed earlier [31]. High blood urea levels have also been reported earlier on the supplementation of the basal diet of buffaloes with NPN compounds [36, 37]. The mean values of serum urea found in this experiment were parallel to the values obtained earlier in buffalo calves fed on ammoniated straw [32]. An increase in the serum creatinine levels is generally seen in degenerative muscle diseases [38]. The quantity of creatinine formed each day depends upon the creatine content of the body, which in turn depends upon the dietary intake, inhibiting the endogenous synthesis rate and the muscle mass [39]. Also, elevated creatinine levels in the serum/plasma are usually associated with various renal diseases. Creatinine is formed during the metabolism of creatine in the muscle and its increased concentrations in the serum is the indicator of a decreased glomerular filtration rate. The cumulative period mean serum creatinine (mg %) values in groups II (2.18) and III (2.24) were comparable and were significantly ($P < 0.001$) higher than those of group I (1.81). This may be attributed to the renal and muscular damages in animals in groups II and III in comparison to group I. However, the mean values of serum creatinine found in this experiment were within the normal range of 1.0 to 2.7 mg %, reported for the domestic animals [30].

Blood serum enzyme activities in different groups at different intervals of time are presented in Table IV. Among various tissues and organs, higher ALP activities occur in the kidneys and intestines, while there are moderate in the liver, lungs, bone, placenta and leukocytes [40]. However, the use of ALP as a diagnostic tool for detecting tissue damage is limited, especially in young growing animals, where bone can be a very significant source of ALP [41]. ALP plays an important role in the regulation of cell division and growth [42] and its activities reach higher levels in the serum of growing animals. Our result revealed that although the serum alkaline phosphatase values were within the normal range, there was a gradual increasing trend in the mean values of serum alkaline phosphatase (ALP), with an increase of age, which could be attributed to the effect of their normal physiological growth. There was a significant ($P < 0.001$) difference in cumulative period mean values of serum ALP activity (KA units), being the highest in group II (28.09), than groups I (26.53) and III (26.31), indicating no adverse effect of the addition of HCl during urea-ammoniation on the activity of serum ALP. On the contrary to this, they [34] did not find any change in serum ALP activity in buffalo bulls fed on urea for a prolonged period. Serum GOT and GPT are both cytoplasmic and mitochondrial enzymes and are distributed in all body tissues, being released by even mild degenerative changes that increase the membrane permeability [41]. However, the highest activities are in the liver, heart, skeletal muscle and erythrocyte. A rise in SGOT activities occurs in acute and occasionally in chronic liver disorders in cattle, but remarkably higher values have been recorded in muscle damage [43]. On the contrary, SGPT is present in very high amounts in the liver and kidney with smaller amounts in the skeletal muscle and heart. Its intracellular location is predominantly cytosolic. Also, trace amounts are present in the pancreas, spleen and lungs. SGPT rises sooner, faster and higher than SGOT in hepatocellular disorders [44]. The transaminase activity is reported to vary with age, productive function and day of collection [30]. Serum GOT and GPT values were also found to be within the normal range, although the cumulative period mean values of SGOT and SGPT in groups II (102.15 and 22.19) and III (103.95 and 23.41) were found to be significantly ($P < 0.001$) higher than that of group I (94.96 and 20.40), which may be due to some adverse effect of long term feeding of ammoniated and HCl treated ammoniated wheat straw. This could be attributed to the chances of

Table IV. Bimonthly serum enzymes activity in buffalo calves fed urea ammoniated wheat straw treated with or without HCl in various groups.

Attributes	Group	Periods (month post feeding)								SEM	Period mean	SEM		Interaction
		0	2	4	6	8	10	12	14			Group	Period	
Serum alkaline phosphatase (KA units)	I	22.24	24.22	26.31	27.12	26.51	27.91	28.40	29.53	2.09	26.53^{y**}	2.21	1.56	NS
	II	22.70	25.06	28.22	30.49	28.50	29.27	29.55	30.96	1.82	28.09^{x}			
	III	19.79	29.81	27/15	27.88	26.53	27.04	27.80	29.51	2.30	26.31^{y}			
Mean***		21.58^{a}	24.70^{ab}	27.23^{bc}	28.49^{bc}	27.18^{bc}	28.06^{bc}	28.58^{c}	30.00^{c}		26.98			
SEM		3.06	2.66	2.85	3.14	0.20	0.27	0.19	0.19					
SGOT (units·mL^{-1})	I	86.03	89.96	95.86	94.99	95.10	97.00	99.93	100.76	1.67	94.96^{x***}	0.95	1.09	NS
	II	88.41	92.85	101.30	101.61	104.88	107.17	108.81	112.20	2.20	102.15^{y}			
	III	89.90	93.72	100.74	102.23	107.71	108.74	113/74	114/81	1.49	103.95^{y}			
Mean***		88.11^{a}	93.72^{b}	99.30^{c}	99.61^{c}	102.56^{d}	104.30^{d}	107.49^{c}	109.25^{e}		100.35			
SEM		1.96	2.01	1.68	2.05	2.15	1.68	1.59	1.67					
SGPT (units·mL^{-1})	I	18.06	18.57	19.45	19.93	19.95	20.87	22.03	24.44	0.43	20.4^{x***}	0.23	0.29	NS
	II	18.01	18.57	21.23	21.91	22.15	23.60	24.77	27.25	0.42	22.19^{y}			
	III	17.93	18.51	21.55	22.04	24.00	26.30	28.33	28.57	0.50	23.41^{y}			
Mean***		17.93^{a}	18.51^{a}	21.55^{b}	22.04^{b}	22.06^{b}	23.59^{b}	25.04^{c}	26.75^{c}		22.00			
SEM		0.46	0.46	0.38	0.34	0.37	0.55	0.50	0.51					

NS: non significant; * $P < 0.05$, ** $P < 0.010$, *** $P < 0.001$; ^{a,b,c,d,e} means with different superscripts in a row differ significantly.
^{x,y,z} Means with different superscripts in a column differ significantly.

Table V. Thyroid hormone activity in the blood serum of buffalo calves fed urea ammoniated wheat straw treated with or without HCl in various groups.

Attributes	Group	Periods (month post feeding)								SEM	Period mean	SEM		Interaction
		0	2	4	6	8	10	12	14			Group	Period	
T_3 ($ng \cdot mL^{-1}$)	I	0.85	1.01	1.01	1.40	1.29	1.20	1.45	`1.61	02.0	1.23	0.20	1.03	NS
	II	0.94	1.14	1.14	1.76	1.57	1.31	1.43	1.56	0.18	1.36			
	III	1.01	1.16	1.16	1.50	1.57	1.69	1.80	2.20	0.08	1.51			
Mean***		0.93^{a}	1.10^{a}	1.10^{a}	1.55^{bc}	1.48^{b}	1.40^{b}	1.56^{bc}	1.79^{c}		1.36			
SEM		0.10	0.13	0.13	0.09	0.13	0.19	0.24	0.20					
T_4 ($ng \cdot mL^{-1}$)	I	18.07	20.79	22.11	23.28	19.57	20.75	21.14	21.48	1.57	20.89^{x}**	1.46	1.03	NS
	II	17.72	20.10	21.28	23.44	23.05	22.20	20.50	23.03	1.01	21.41^{x}			
	III	18.36	19.74	22.45	23.86	22.38	28.68	23.51	22.93	0.96	22.74^{y}			
Mean***		18.05^{a}	20.21^{b}	21.95^{bc}	23.53^{c}	21.67^{bc}	23.88^{c}	21.72^{bc}	22.48^{bc}		21.68			
SEM		0.90	0.74	0.90	0.92	1.50	1.92	1.52	1.47					

NS: non significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ^{a,b,c} means with different superscripts in a row differ significantly.
^{x,y,z} Means with different superscripts in a column differ significantly.

some pathological alterations in some vital organs of buffalo calves of groups II and III. The thyroid hormones have been shown to control many physiological functions including cellular metabolism [45]. It is well known that T_3 is physiologically more active than T_4, and provides a better indication of the metabolic status of the animal [46]. Thus the serum T_3 and T_4 concentrations seem to be significantly related to the growth and age of the animal. The mean values of serum T_3 and T_4 found in this experiment were within the normal physiological range (Tab. V). The cumulative period mean serum T_3 ($ng{\cdot}mL^{-1}$) values were similar in all three groups. However, the values of T_4 ($ng{\cdot}mL^{-1}$) were significantly ($P < 0.01$) higher in group III (22.74) as compared to groups I (20.89) and II (21.41). This indicated a higher basal metabolic rate in group III as compared to groups I and II. The effects of thyroid hormones are increasing the basal metabolic rate, making more glucose available to meet the elevated metabolic demands by increasing glycolysis, gluconeogenesis and glucose absorption from the intestine, stimulating new protein synthesis, increasing lipid metabolism and conversion of cholesterol into bile acids and other substances, activation of lipoprotein lipase and increasing the sensitivity of adipose tissue to lipolysis by other hormones, stimulating the heart rate, cardiac output and blood flow and increasing neural transmission, cerebration and neuronal development in young animals [47].

4. CONCLUSION

The mean values of all the blood parameters varied between the groups but they were with in the normal physiological range in all of the groups. Therefore, it may be concluded that long term feeding of ammoniated wheat straw treated with and without HCl to growing male buffalo calves does not have any adverse effect on blood biochemical parameters in growing male buffalo calves.

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