

EFFECTS OF AMMONIATED RICE STRAW ON HEALTH, RUMEN LIQUOR AND BLOOD IN HEIFERS AND RAMS.

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SUMMARY

Thirty animals (16 heifers and 14 rams) were used in this study. The first group comprised of 16 heifers (2 years old, 400 ± 10 kg weight). The heifers, were subdivided into 8 tested and 8 control. The tested heifers were fed on ammoniated rice straw 3 % for 6 months and concentrate mixture, however the control heifers fed on ration with rice straw, berseem and concentrate mixture. The second group comprised of 14 fattened rams (1-1.5 years old, 60 ± 10 kg weight). The fattened rams were subdivided into 7 tested and 7 control. The tested rams fed on ammoniated rice straw 3 % and concentrate mixture for 10 months, while the controls were kept on rice straw, berseem with concentrate mixture. The clinical signs of bonkers syndrome were not manifested on tested heifers and rams. The rumen liquor pH revealed insignificant increase in tested heifers and significant increase ($P < 0.01$) in tested rams, the rumen liquor

ammonia showed significant increase in tested rams and the ruminal total volatile fatty acids, were significantly increased ($P < 0.05$) in tested heifers and non-significantly increased in tested rams in comparison to the controls.

The blood ammonia level showed significant increase in tested heifers ($P < 0.001$) and in tested rams ($P < 0.01$).

Insignificant increase in serum urea and creatinine in tested heifers and rams. Non-significant increase in serum total bilirubin in tested heifers and significant increase ($P < 0.01$) in tested rams. The serum level of ALT (GPT) revealed significant increase in tested heifers ($P < 0.05$) and in tested rams ($P < 0.01$), while the serum level of AST (GOT) was significantly increased ($P < 0.001$) in tested heifers and non-significantly decreased in tested rams. Non significant alterations were reported in serum choles-

terol, calcium, phosphorus and magnesium in both tested heifers and rams versus the controls. The serum proteinogram in tested heifers showed significant decrease in total protein ($P < 0.05$) and albumin ($P < 0.001$) while non-significant increase in globulin, however, in tested rams, there was significant increase in total protein, albumin ($P < 0.001$) and globulin ($P < 0.05$). The serum glucose level was significantly increased in tested rams in relation to the controls. The erythrogram showed non significant increase in hemoglobin, hematocrit and RBCs in tested heifers and significant decrease in hemoglobin ($P < 0.01$) and RBCs ($P < 0.05$), while insignificant decrease in hematocrit in tested rams. The leukogram of the tested heifers and rams showed non-significant fluctuations in comparison to the controls. It can be concluded that, inspite of the economical use of ammoniated rice straw in the feeding of heifers and rams, unfortunately, it causes hepatocellular damage and anemia, so, further studies were needed to overcome these bad effects.

INTRODUCTON

Recent trends in animal nutrition aim to improve the quality of the available roughages and agriculture residues (Abdel - Gawad et al. 1989, Fahmi, 1990, Hari-Singh et al., 2001 and Takenaka et al., 2002). Ammoniation of rice straw is midely used by livestock producers in

Egypt (El-Sayes, 1993 and Ebrahim, 2002). A comprehensive research work has been done concerning the effect of ammonia treatment in improving the digestibility, feeding efficiency and productivity of ruminants (Fahmi 1990, Farghaly, 1993 and Wiedmeier et al., 2002).

On the contrary, the effect of ammonia treatment of the straw on intermediary metabolism, different organ function and health condition of fed animals were not fully investigated, although a great deal of problems were set up as a result of feeding such rations (El-Kapani et al., 1985, Kubesy, 1987, El-Hamamsy et al., 1990, Kubesy et al., 1997, Saleh et al., 1998 and Hassoun et al., 2002).

The aim of this work is to investigate the effect of ammoniated rice straw on some ruminal, blood biochemical constituents and hematological findings in heifers and rams.

MATERIALS AND METHODS

Animal and rations:

Thirty heifers and rams were used in this study. Heifers and rams were healthy based upon clinical and laboratory examinations. They were divided into two groups: the first group comprised of 16 heifers (2 years old, 400 ± 10 kg weight). This group was subdivided into 8 tested heifers and 8 control. The tested heifers were fed on ammoniated rice straw 3 % for 6 months with berseem and

concentrate mixture, while the controls were kept on rice straw, berseem and concentrate mixture only. The concentrate mixture composed of wheat bran (35 %), corn (25 %), cotton seed cake (20 %), molasses (7.85 %), rice hulls (7 %), urea (0.8 %), sulphur (0.15 %), dicalcium phosphate (3 %) and common salt (1.2 %). The heifers were located in a research farm in Alexandria governorate. The second group comprised of 14 fattened rams (1-1.5 years old, 60 ± 10 kg weight). This group was subdivided into 7 tested rams and 7 control. The tested rams fed on ammoniated rice straw 3 % with berseem and concentrate mixture for 10 months, while the controls were kept on rice straw, berseem and concentrate mixture. The concentrate mixture (El-Muffeed) contained 2.5 % urea and minerals mixture. The fattened rams were located in a private farm in Fayoum governorate. Ammoniation of rice straw with anhydrous ammonia (3 % on dry matter basis) followed the stack method (Sundstol et al., 1978). The rations offered to heifers and rams were formulated to be isocaloric and isonitrogenous and the requirement calculated according to NRC (1978) allowance. The non protein nitrogen content of the ammoniated rice straw containing ration was within the recommended permissible level as formulated according to NRC (1978) allowance. Clinical examination was carried out according to standard methods described by Rosenberger (1979) and Radostitis et al., (2000).

Blood and rumen liquor samples:

The blood and rumen liquor samples were collected every month through the last 3 months of the experiment from each animal in both first and second groups. Rumen liquor samples were taken 2 hours post feeding and examined by standard methods for pH (using digital pH meter, Orion model 299A), ammonia (Okuda et al., 1965 and modified by McCullough, 1967) and total volatile fatty acids (Eadie et al., 1967). Blood plasma ammonia level was estimated by standard method (Okuda et al., 1965 and modified by McCullough, 1967). Blood serum samples were used to determine the levels of urea (Fawcett and Scott, 1960), creatinine (Bartel, 1972), bilirubin (Walters and Gerarde, 1970), alanine aminotransferase (ALT or GPT) and aspartate aminotransferase (AST or GOT) (IFCC, 1976), cholesterol (Allain, 1974), total protein (Weichselbaum, 1946), albumin (Drupt, 1974), globulin by subtraction, glucose (Trinder, 1969), calcium (Corns and Ludman, 1987), inorganic phosphorus (Erthingshausen and Daly, 1972) and magnesium (Gindler, 1971). Whole blood samples with EDTA were analyzed for determination of hemoglobin, hematocrit, RBCs, total and differential leukocytic counts (Coles, 1986).

Statistical analysis:

The obtained data were statistically analyzed according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Clinical findings:

Bovine bonkers (Morgan and Edwards, 1986) is a hyperexcitability syndrome reported in cattle and calves that has been fed ammoniated molasses, hay and silage, and molasses-urea protein block (Haliburton and Morgan 1989). Cattle develop a syndrome of hyperexcitability manifested by restlessness, rapid blinking, dilation of pupils, impairment of vision, involuntary ear twitching, frequent urination and defecation, rapid respiration, trembling, loss of balance, salivation, frothing at the mouth, sweating, bellowing, and most markedly, sudden stampeding, involving galloping in circles and colliding with fences or other animals (Perdok and Leng, 1985). Signs of forestomach dysfunction such as rumen hypomotility, bloat, and abdominal pain are also present. In milder cases, decreased appetite, rumen hypomotility, recurrent tympany and diarrhea may be the most prominent signs. Thus the syndrome may appear as a form of forestomach disease (Garry, 2002). These symptoms have been reported in adult cows, bulls, yearlings, calves and sheep (LaBore et al., 1985). Careful clinical examination of heifers and rams throughout this study revealed that feeding for 6 months in heifers and for 10 months in rams on ration containing ammoniated rice straw 3 % has no adverse effects on animal health. This opinion is supported and agreed with the findings of Khadr (1995), Kubesy et al.,

(1997) and Saleh et al., (1998) in sheep, cows and buffaloes, respectively, fed on rations containing ammoniated rice straw 3 % for years. Hendrex and Lemenager (1984) mentioned that in many instances of ammoniated feed toxicity, the associated forages contained ample soluble sugars and greater than 20 per cent moisture, and received excessive rates of anhydrous ammonia application (greater than 3 per cent of the forage dry matter). In this study the ammoniated rice straw with 3 % ammonia and suitable quantity of molasses.

Rumen liquor parameters:

The study revealed insignificant increase in ruminal pH in tested heifers and significant increase ($P < 0.01$) in tested rams when compared with the control ones (Table 1). The result in heifers were in agreement with Garret et al., (1997). This opinion was supported by the findings of Garry (2002), who mentioned that the pH on feeding ammoniated straw usually does not increase above neutral because these rations contain sufficient amounts of readily fermentable carbohydrate to maintain a slight acidic pH. In rams, the result was similar to that of Khalel (1994) and Khadr (1995). Cordesse and Taba-Tabai (1981) found that the pH of ruminal contents of lambs fed ammoniated straw was between 6 and 7.

The present data indicated that the ruminal ammonia concentration showed significant increase ($P < 0.05$) in tested heifers and non-significant in-

crease in tested rams than controls (Table 1). These results were in harmony with those of Andrietto and Cavall (1988) who reported that treatment of straw with ammonia lead to increase in ammonia level in the ruminal fluid and also in agreement with Bartley et al., (1976) who stated that high concentration of ruminal ammonia does not necessarily indicate ammonia toxicity.

Regarding the ruminal total volatile fatty acids, there was significant increase increase ($P < 0.05$) in tested heifers and non-significant increase in tested rams versus the control (Table 1). These results were similar to those of Horton (1978) and Rakha (1985). Mehra et al. (1983), also agreed with these results and found that total bacterial mass and production of volatile fatty acids was more in feeds with ammoniated straw.

Blood biochemical parameters:

The blood ammonia level showed significant increase in tested heifers ($P < 0.001$) and in tested rams ($P < 0.01$) in comparison to the controls (Table 2). This significant increase in blood ammonia level was attributed to feeding on 3 % ammoniated rice straw. These results inspite of being statistically significant yet from the toxicological view, it is considered insignificant clinically because the clinical signs of ammonia toxicity (Bonkers syndrome) were not appeared. Word et al., (1969) stated that toxicity symptoms appeared when ammonia level averaged 1.0 mg/dl. Furthermore, Bartley et al., (1976) reported that clinical signs of toxicity appear in cattle when blood ammonia bypassing hepatic circulation rather than the inability of the liver to metabolize incoming ammonia. These results were in agreement with Roller et al., (1982), Lloyd (1986), Haliburton and Morgan (1989), El-Hamamsy et al., (1990) and Moawad (1997).

Table (1): Effect of feeding on rations containing ammoniated rice straw on some rumen liquor parameters in heifers and rams (mean value \pm standard error).

Parameters	Heifers		Rams	
	Control	Tested	Control	Tested
pH	6.6 \pm 0.4	6.8 \pm 0.5	6.3 \pm 0.5	6.9 \pm 0.2**
Ammonia (mg/dl)	18.1 \pm 8.4	27.6 \pm 6.6*	21.6 \pm 9.1	30.1 \pm 5.2
Total volatile Fatty acids (mEq/dl)	6.5 \pm 1.7	8.1 \pm 1.1*	9.6 \pm 0.9	10.3 \pm 2.1

* Significant $P < 0.05$

** Significant $P < 0.01$

Statistical analysis showed insignificant increase in serum urea and creatinine in tested heifers and tested rams in relation to the controls (Table 2). These results were in harmony with Kubesy (1987), El-Saadany (1991), Khalel (1994) and Kubesy et al., (1997). Visek (1972) stated that the absorbed ruminal ammonia is transported by the portal vein to the liver where it is converted to urea. Also, there was direct relationship between the serum urea and the ruminal ammonia levels.

The serum total bilirubin showed non-significant increase in tested heifers and significant increase ($P < 0.01$) in tested rams than controls (Table 2). This increase may be attributed to the occurrence of hepatopathy in tested heifers and rams on rations supplemented with ammoniated rice straw for 6 months and 10 months, respectively. Liver failure in ruminants, particularly chronic liver failure, is associated with a much less striking elevation in serum bilirubin than occurs in the horse (Abdou 1976, Carlson, 2002). These results agreed with Roller et al., (1982) and Duncan and Prasse (1986).

Regarding the serum level of ALT (GPT), revealed significant increase in tested heifers ($P < 0.05$) and in tested rams ($P < 0.01$) in comparison to the control groups. While, the serum level of AST (GOT) was significantly increased ($P < 0.001$) in tested heifers and non-significantly in-

creased in tested rams in relation to the controls (Table 2). Duncan and Prasse (1986) mentioned that the serum ALT (GPT) is more specific to liver than AST (GOT). The increase of ALT activity was went parallel with AST activity supporting the occurrence of hepatopathy in tested heifers. Also, in tested rams the use of ammoniated rice straw plus El-Muffeed which contained 2.5 % urea for 10 months may cause liver damage. These results were in harmony with those of Davidovich et al., (1977), Amin and El-Saifi (1980), Emmanuel et al., (1982), El-Bedawy et al., (1989) El-Hamamsy et al., (1990), Kubesy et al., (1997). The rations containing ammoniated rice straw and El-Muffeed showed higher glutamate pyruvate transaminase (GPT or ALT) levels, however, ammonia treatment of rice straw and/or El-Muffeed supplementation had non-significant effect on AST or GOT and alkaline phosphatase (AP) (Khalel, 1994). On the other hand, Khadr (1995) reported insignificant changes in serum levels of total bilirubin, ALT and AST in sheep fed ration containing ammoniated rice straw 3 % for 191 days.

Non-significant alterations were reported in the serum cholesterol level in tested heifers and rams versus the control (Table 2).

Table (2): Effect of rations containing ammoniated rice straw on plasma ammonia and some serum biochemical constituents in heifers and rams (mean value \pm standard errors).

Parameters	Heifers		Rams	
	Control	Tested	Control	Tested
Plasma ammonia (mg/dl)	0.06 \pm 0.005	0.16 \pm *** 0.005	0.1 \pm 0.009	0.17 \pm ** 0.02
Urea (mg/dl)	21.8 \pm 1.2	30.3 \pm 4.0	23.2 \pm 1.6	29.5 \pm 4.1
Creatinine (mg/dl)	1.12 \pm 0.12	1.6 \pm 0.2	1.45 \pm 0.1	1.57 \pm 0.2
Bilirubin (mg/dl)	0.4 \pm 0.02	0.53 \pm 0.06	0.29 \pm 0.04	0.47 \pm ***0.04
ALT (GPT) (U/L)	32.5 \pm 2.6	41.6 \pm * 3.2	73.8 \pm 3.6	92.5 \pm ** 4.2
AST (GOT) (U/L)	50.1 \pm 2.2	89.6 \pm *** 5.8	114.5 \pm 4.6	114.8 \pm 11.4
Cholesterol (mg/dl)	122.4 \pm 8.2	114.3 \pm 9.1	102.7 \pm 5.2	105.0 \pm 1.9
Total protein (g/dl)	8.6 \pm 0.04	8.2 \pm *0.16	6.65 \pm 0.13	9.01 \pm *** 0.18
Albumin (g/dl)	4.1 \pm 0.08	3.4 \pm *** 0.12	2.53 \pm 0.13	4.42 \pm *** 0.16
Globulin (g/dl)	4.5 \pm 0.09	4.8 \pm 0.11	4.12 \pm 0.12	4.6 \pm *0.15
Glucose (mg/dl)	51.6 \pm 2.8	75.4 \pm *** 4.5	62.3 \pm 2.3	69.7 \pm 6.6
Calcium (mg/dl)	9.2 \pm 0.21	9.1 \pm 0.24	10.4 \pm 0.3	8.9 \pm 0.4
Phosphorus (mg/dl)	4.1 \pm 0.1	4.0 \pm 0.1	5.5 \pm 2.0	4.5 \pm 2.0
Magnesium (mg/dl)	4.1 \pm 0.007	3.5 \pm 0.2	2.4 \pm 0.08	3.0 \pm 0.6

* = Significant at P < 0.05

*** = Significant at P < 0.001

** = Significant at P < 0.01

Concerning the serum proteinogram profile in tested heifers there was significant decrease in total protein (P < 0.05), and albumin (P < 0.001), while non-significant increase in globulin, however in tested rams there was signifi-

cant increase in total protein, albumin (P < 0.001) and globulin (P < 0.05), in comparison to the controls (Table 2). The significant decrease in tested heifers total protein and albumin was a result of decrease the efficiency of the liver to

synthesize albumin due to feeding on ammoniated straw. These results were in agreement with those of El-Saadany (1991) and Kubesy et al. (1997) in lactating cows with urea supplementation or ammoniation of rice straw, on the other hand, Vengrin et al., (1981) found that replacing up to 34 % of digestible protein in the diet for fattening young bulls with any form of urea, did not lower the concentration of total protein. The significant increase in total protein, albumin and globulin in tested rams may be due to feeding on ammoniated rice straw. These results were in harmony with those of Kubesy (1987), Mohamed (1989) and Moawad (1997) in sheep with urea supplementation or ammoniation of rice straw, who also mentioned that longterm feeding of ammoniated rice straw can produce immune response. This immune response represented by antibodies production and consequently the serum globulin. This opinion was supported by the findings of Targowski et al., (1984), Kluoiniski et al., (1984) and Khadr (1995). On the other hand, these results disagreed with El-Bedawy et al., (1989), Farghaly (1993) and Yackout (1993) who recorded that plasma total protein, albumin and globulin (g/dl) of goat fed ammoniated wheat straw were 8.22, 3.57 and 4.68, whereas for those fed non-ammoniated were 8.28, 3.53 and 4.47, respectively.

The serum glucose level showed significant increase in tested heifers ($P < 0.001$) and non-

significant increase in tested rams versus the controls (Table 2). These results were similar to those of Amin and El-Saifi (1980), Emmanuel and Edjtehadi (1981), Rakha (1985) and El-Hamamsy et al., (1990), and disagreed with Allen et al., (1993) and Kubesy et al., (1997). Emmanuel et al., (1982) suggested that hyperglycemia could not possibly result from enhanced gluconeogenesis but from enhanced glycogenolysis. However, Emmanuel and Edjtehadi (1981) reported that the impaired utilization of glucose in urea or ammonia treated animals can result from inhibitory effect of ammonia on synthesis or secretion of insulin, or damage of the cell membrane, resulting in adverse effect on glucose transport and utilization or inhibition of Krebs cycle and oxidative phosphorylation.

Statistical analysis revealed insignificant alterations in the serum levels of calcium, phosphorus and magnesium between the control and the tested groups (Table 2). These results were in agreement with Ochodnicky and Pilko (1973), Ochrimenko et al., (1987) Allen et al., (1993) and Khadr (1995). On the other hand, Sharma et al., (1973) found that there was significant decrease in serum phosphorus in lactating cows due to feeding urea and ammonia bicarbonate, and Kubesy et al., (1997) found that the serum calcium and magnesium levels were significantly decreased ($P < 0.05$) while serum phosphorus was non-significantly affected by urea and ammonia

containing ration in lactating cows.

Hematological findings:

The erythrogram showed non-significant increase in hemoglobin, hematocrit and RBCs in tested heifers and significant decrease in hemoglobin ($P < 0.01$), and RBCs ($P < 0.05$), while non-significant decrease in hematocrit in tested rams in relation to the controls (Table 3). These results were agreed with Sheik et al., (1986), and Kubesy (1987). Duncan and Prasse (1986) stated that the exposure to toxic chemicals (anhydrous ammonia in this study) may be the cause of this anemia in tested rams.

The leukogram of the tested heifers and rams showed non-significant fluctuations in comparison to the controls (Table 3). Similar leukograms were reported by Pilko and Ochodnický (1973), Rakha (1985), Shiek et al., (1986), and Kubesy (1987). It can be concluded that, in spite of the economical use of ammoniated rice straw in feeding of heifers and rams, unfortunately, it causes hepatocellular damage and anemia. So., further studies were needed to overcome these bad effects.

Table (3): Effect of rations containing ammoniated rice straw on some hematological findings in heifers and rams (mean value \pm standard error).

Parameters	Heifers		Rams	
	Control	Tested	Control	Tested
Hemoglobin (g%)	8.7 \pm 0.7	8.9 \pm 0.4	11.1 \pm 0.6	7.3** \pm 1.4
Hematocrit (vol%)	33.5 \pm 0.9	34.4 \pm 0.9	34.5 \pm 2.2	28 \pm 2.8
RBCs ($\times 10^6/\mu\text{l}$)	5.3 \pm 0.4	6.5 \pm 0.8	11.3 \pm 0.9	7.9 \pm 1.1*
WBCs ($\times 10^3/\mu\text{l}$)	8.06 \pm 0.6	8.15 \pm 0.4	7.7 \pm 0.8	10.2 \pm 1.0
Neutrophils (%)	39.1 \pm 3.5	30.9 \pm 3.6	35.4 \pm 3.1	28.8 \pm 4.5
Lymphocytes (%)	59.3 \pm 3.7	66.4 \pm 3.5	62.8 \pm 3.9	69.0 \pm 4.2
Esinophils (%)	0.63 \pm 0.3	0.5 \pm 0.3	1.7 \pm 0.8	0.8 \pm 0.4
Monocytes (%)	1.0 \pm 0.5	1.8 \pm 0.6	1.5 \pm 0.8	1.2 \pm 0.6
Basophils(%)	0.0 \pm 0.0	0.0 \pm 0.0	0.3 \pm 0.2	0.0 \pm 0.0

* = Significant at $P < 0.05$

** = Significant at $P < 0.01$

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