THE FLUCTUATION EFFECT OF ATMOSPHERIC AMMONIA (NH_3) EXPOSURE AND MICROCLIMATE ON HEREFORD BULLS HEMATOCHEMICAL

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ABSTRACT

Ten Hereford bulls housed indoors in individual tie stalls were used to explore the relationships atmospheric ammonia exposure and microclimate on beef cattle hematochemical, and to know effect and to prediction equations the atmospheric ammonia exposure and microclimate with various hematochemical parameters. This study was conducted in Animal Station, Department of Agricultural Biotechnology, Seoul National University (SNU), Seoul, South Korea, for 40 days during summer. Results of this study indicated that there were negative correlation between ammonia with temperature and positive correlation between ammonia with humidity. There were significantly negative effect of ammonia on hematochemical, except neutrophyls and ratio of neutrophyls to lymphocytes. Simultaneous effect between ammonia, temperature and humidity, mainly effected on glucose, hexokinase, lymphocytes, neutrophyls, N:L Ratio, and lactate. Simultaneous effect between ammonia, temperature and humidity proved to be a good indicator for predicting the profile of glucose, hexokinase, lymphocytes, neutrophyls, N:L Ratio in the blood.

Keywords : Ammonia, Hematochemical, Hereford, Humidity, Temperature

INTRODUCTION

Over the past 15 years, environmental concerns related to animal agriculture have focused on improvements in manure management to mitigate runoff and pollution of lakes, streams, and other surface waters (Moody and Burns, 2006). The main factors that affect ammonia N loss are housing and manure-handling strategies, diets, bedding type, barn ventilation, and temperature (Powel *et al*., 2008) and humidity (Hongqing, 2010). Confinement housing and atmospheric pollution are becoming increasingly important factors in animal agriculture and ammonia is one of the major irritant gases involved.

Atmospheric ammonia (NH_3) has been shown to be detrimental to animal health and performance (Carlile, 1984; Kristensen and Wathes, 2000) and is cited as an environmental concern as well (NRC, 2003; USDA, 2005). Exposure to high concentrations of NH_3 (>50 ppm) causes keratoconjunctivitis, with symptoms including watery eyes, closed eyelids, rubbing of eyes with the wings, and blindness (Bullis *et al*., 1950; Faddoul and Ringrose, 1950).

The temperature as the main factor can affect the health of sows and humidity plays the role on the heat transfer. The concentration of ammonia emissions influenced by temperature and relative humidity simultaneous.

The physical, chemical properties of ammonia, its sources and detoxification, its effects in biological systems, its influence upon insulin action and glucose metabolism, and its possible effects on reproduction (Rigout *et al*., 2002). At physiological pH, nonionic ammonia concentrations remain low but are primarily responsible for toxic effects. Thus, biologically significant changes of ammonia concentrations may not be revealed by determinations of ammonia in blood plasma. For these and other reasons the sub acute toxicity of ammonia often is unrecognized, and its effects on intermediary metabolism and the hormonal in normal and disease states remain poorly understood. Effects of ammonia may be stimulatory at low

concentrations and inhibitory as concentrations rise or exposure is extended.

Exposure of ammonia continuously caused decreases of cyclic adenin monophospate (cAMP) in the olfactory system, so increase in both respiration rate and respiratory minute volume. This phenomenon is as consequence damage blood function and metabolism, specially of glucose utilization. Most of the previous experiment on ammonia (NH_3) toxicity for animals has been related to general symptomatic effects, however, a limited number of investigations have been concerned with hematochemical.

The objectives of this research was to explore the relationships atmospheric ammonia exposure and microclimate on beef cattle hematochemical, and to know effect and to prediction equations the atmospheric ammonia exposure and microclimate with various hematochemical parameters.

MATERIALS AND METHODS

Animal, Housing, and sampling

This study was conducted in Animal Station, Department of Agricultural Biotechnology, Seoul National University (SNU), Seoul, South Korea. Ten Hereford bulls (BW average 987 ± 23 kg) were housed indoors in individual tie stalls. Blood sampling and measurement of ammonia, temperature, and humidity were conducted simultaneously in the housing, at early morning (05.00 AM) and afternoon (17.00) once a 4 days for 40 days.

Methods of Samples Analysis

Ammonia emissions, atmospheric ammonia exposure in the housing was determined using an indophenols method (Rotz and Oenema, 2006). The indophenols method for determining ammonia in air samples was based on the formation of an indophenols blue pigment during the reaction of phenol and hypochlorite in the presence of ammonia. In an alkaline medium (pH = 8.0 – 11.5) a chloramine is first obatined. This then reacts with the surplus hydochlorite and with the phenol forms quinone chloramine in the presence of catalytic quantities of sodium nitroprusside. Quinone chloramine further reacts with surplus phenol to produce indophenol. The blue coloration is due to the dissociated form of indophenol. 20 mL borid acid 0.5% was placed into adsorption bottles of each and connect 2 adsorption bottles. Absorb the gas for 3 minutes with the flow rate of 2.0 L/min. The adsorbent and rinse the adsorption bottles with boric acid twice were collected and then 10 mL sample solutions and diluted standard solution (0, 0.2, 0,4. 0.6, and 0.8) were into the new tubes. Then 5 mL solution B (contain phenol and sodium nitroprusside) and 5 mL solution C (contain NaOCl, NaOH) and $Na_2HPO_4.12H_2O$) were added in each of tubes. After one hour, absorbance was read of each sample versus a blank by UV-1601 Spectrophotometer at 640 nm wavelength.

Temperature and humidity. Temperature and humidity in the housing were measured, respectively by digital thermometer and hygrometer. The thermometer and hygrometer were each placed at the centre, right and left inside housed.

Hematologic. Hematologic analyses were included of eritrocyte, hemoglobin, lympocyte, neutophyl was determined using by hematology analyzer. Total RNA Reticulocytes were enumerated per 1000 erythrocytes in blood smears vitally stained with brilliant cresyl blue and counterstained with Wright's stain.

Glucose. Glucose concentration of the blood plasma was determined using glucose oxidase/peroxidase enzyme and dianisidine dihydrochloride (Sigma) as described previously (Regmi *et al*., 2008), and the absorbance was determined with a microplate spectrophotometer (UV-1601). Net glucose disappearance was calculated by subtracting the amount of glucose in incubation media of treatment flasks from that of 0-min control.

Lactate. Lactate concentration in the blood was determined using a lactate assay kit, as described previously (Acosta *et al*., 2010). Whole blood were homogenized in 0.9% saline and centrifuged at 2,800 *g* for 6 min, and the supernatant was diluted to 1:7 with saline. Then 50 mL of both samples and the lactate standards were added in duplicate to a 96-well microplate. An equal volume of a reaction mix containing the lactate assay buffer, lactate probe, and enzyme mix was added to each of the wells. The plate was incubated at 37°C for 30 min in darkness, and absorbance was read in a microplate reader at 565 nm wavelength.

Hexokinase and Pyruvate Kinase. The blood plasma was used for the measurement of hexokinase (EC 2.7.1.1) and pyruvate kinase (EC

2.7.1.40) activity as described by Darrow and Colowick (1962) and Cardenas and Dyson (1973), respectively. Briefly, for the glucokinase assay, 2.5 mL of a reaction cocktail (pH 8.5; prepared by mixing 5.0 mL of 100 mM glycylglycine buffer, 5.0 mL of 200 mM ATP, 6.0 mL of 0.01% cresol red, and 33.4 mL of deionized water) was added with 0.4 mL of 200 m*M* glucose and 0.1 mL of the supernatant in a cuvette, and the absorbance of the solution was measured at 560 nm at 25°C for 5 min using a spectrophotometer (Beckman DU50, Beckman Analytical, Palo Alto, CA). For the pyruvate kinase assay, a reaction cocktail composed of 1.3 mL of deionized water, 0.8 mL of 100 mM potassium phosphate buffer (pH 7.6 at 37°C), 0.16 mL of 8 mM phosphoenol pyruvate, 0.2 mL of 3 mM β-NADH, 0.2 mL of 100 mM MgSO4, 0.1 mL of 40 mM ADP, 0.04 mL of l-lactic dehydrogenase, and 0.1 mL of 30 mM fructose diphosphate was prepared. The cocktail was mixed with 0.1 mL of the supernatant in a cuvette, and the absorbance of the solution was determined at 340 nm at 37°C for 5 min using the same spectrophotometer.

Statistical Analysis

Microsoft Office Excel 2007 was used to analyze the experimental data and for graphing purposes (regression equations). Box plots were constructed to provide a visual summary of the distribution chematochemical data. Pearson product–moment correlation coefficients were calculated by choosing bivariate with two tailed option using SPSS 12 version (SPSS, 2004) to show the relationship between the atmospheric ammonia and microclimate with chematochemical parameters.

RESULTS AND DISCUSSION

Person correlation test for ammonia (NH_3), microclimate, and hematochemical, showed in Table 1 and simultaneous effect and prediction equations of atmospheric ammonia and microclimate on hereford bulls chematochemical, showed in Table 2.

Atmospheric Ammonia Exposure and Microclimate

Pearson correlation tests for microclimate, namely temperature and humidity showed a negative and positive correlation with ammonia (Table 1) at r = -0.798 and 0.879, respectively. Negative correlation between ammonia with temperature and positive correlation between ammonia with humidity were observed in the whole day ($P<0.05$). The significantly negative and positive correlation were similar with the experiment result by Miller e*t al.* (2003) and Weeb *et al.* (2004). However, significantly positive correlation between ammonia with temperature was observed by Kim *et al*. (2005). The contrary results suggest that the phenomenon is complicated, and likely depends on the relationship humidity and airborne particles, the water-carrying capacity of air as it relates to temperature, and affinity of water molecules to odorous compounds.

Hematochemical

Pearson correlation tests for relationship between ammonia and microclimate with hematochemical (Table 1) indicated that there were significantly negative effect of ammonia on hematochemical, except neutrophyl and ratio of neutrophyl to lymphocytes. Although, correlation of ammonia with neutrophyl and ratio of neutrophyl to lymphocytes were positive, but indicates a stress of metabolism and nervous system in Hereford bulls. The effect of ammonia emission on metabolism has reported (van Duinkerken *et al*., 2005), and equilibrium function damage (Starmans, 2007), olfactory nervous system and cellular metabolism (Nelson and Cox, 2008).

Positive correlation of temperature with hematochemical (except N:L Ratio) was likely there are were simultaneous effect between ammonia and humidity plays the role on the heat transfer to carry out ammonia.

Simultaneous effect of atmospheric ammonia exposure and microclimate with various hematochemical parameters has predicted by prediction equation (regression equation), and the effect proportion of ammonia and microclimate on hematochemical were analyzed by determination index (R^2) (Table 2).

The greatest effect by simultaneous effect of atmospheric ammonia exposure and microclimate on hematochemical parameters were recorded during study were simultaneous effect of ammonia and temperature on glucose concentration in blood was 0.87 or 87% , and glucose level in the blood can estimated by prediction equation was Glucose = 16.85-0.67A+0.01T (Table 2 and Figure 1). This

Table 1. Person Correlation (r) Test for Ammonia (NH_3), Microclimate, and Hematochemical

	Am	Temp	Hum	Ery	Hb	Lym	Neu	N:L Ratio	% RNA rt	Glu	Lac	Hex	PK
Am	1	-0.8	0.88	-0.81	-0.62	-0.69	0.67	0.5	-0.51	-0.94	-0.54	-0.61	-0.6
Temp		1	-0.94	0.71	0.39	0.89	-0.85	-0.8	0.48	0.75	0.45	0.76	0.23
Hum			1	-0.73	-0.64	-0.82	0.84	0.74	-0.53	-0.86	-0.65	-0.78	-0.47
Ery				1	0.31	0.52	-0.37	-0.43	0.4	0.63	0.15	0.43	0.23
Hb					1	0.33	-0.52	-0.28	0.26	0.7	0.86	0.46	0.89
Lym						1	-0.83	-0.74	0.66	0.77	0.54	0.73	0.26
Neu							1	0.88	-0.61	-0.77	-0.62	-0.62	-0.33
N:L ratio								1	-0.66	-0.54	-0.3	-0.41	-0.04
% RNA rt									1	0.58	0.35	0.33	0.17
Glu										1	0.72	0.59	0.72
Lac											1	0.67	0.75
Hex												1	0.27
PK													1

All correlations is significant at the 0.05 level

Am	: Ammonia	Hb	: Hemoglobin	Glu	:Glucose
Temp	: Temperature	Lym	: Lymphocytes	Lac	: Lactate
Hum	: Humidity	Neu	: Neutrophyls	Hex	: Hexokinase
Ery	: Erythrocytes	%RNA rt	: %RNA reticulocytes	PK	: Pyruvate Kinase

prediction equation indicated that glucose was reduced 1 nm/L if ammonia increased 0.67 ppm or glucose was increased 1 nm/L if ammonia reduced 0.67 ppm, likewise on temperature. Similarly, the other parameters can estimated by prediction equations (Tabel 2).

Generally, results this study by prediction equations showed that ammonia, humidity and temperature increased causes hemathochemicals decreased. Previous studies (Amon *et al*., 2001; Anderson *et al*., 3002; Misselbrook *et al*., 2005; and Powell *et al*., 2008) showed that differences in temperature and relative humidity, which would affect reactive ammonia emissions. Atmospheric ammonia exposure in the housing continuously caused decreases of cyclic adenin monophospate (cAMP) in the olcatory system.

Ammonia arrives at the mucous layer and binds directly to an olfactory receptors is longer present causes Ca^{2+} reduces the affinity of the cation channel fo cAMP (Nelson and Cox, 2008). cAMP in olfactory affect to central nervous system (Starmans, 2007). Ammonia toxicity is signaled most often by abnormal neurologic behavior, much research has emphasized effects of ammonia on the central nervous system. Visek (1984) reported that a commonly expressed hypothesis was that ammonia toxicity increased glutamate formation depletes the TCA cycle of a-ketoglutarate to interrupt energy metabolism and ATP synthesis. This phenomenon have been reduces commonly hematochemical profile.

The specific objective of the current study indicate that ammonia concentration in housing showed a negative correlation on the hemoglobin. If ammonia concentration exposure increased was reduced hemoglobin level in the blood. Hemoglobin plays the role on oxygen transport to cells. A low level of oxygen in the cells caused metabolism damaged, specially glucose metabolism and reduced nutrient absorption in the intestine. Because toxicity is signaled most often by abnormal neurologic behavior, much research has emphasized effects of ammonia on the central nervous system.

The present study showed a reducing glucose metabolism, shown at Table 1 with a negative correlation index on hexokinase (-0.607) and Pyruvate Kinase (-0.600). Visek (1984) and El-Kadi *et al*. (2006) reported that a commonly expressed hypothesis is that increased glutamate formation depletes the TCA cycle of a-

Table 2. Simultaneous Effect and Prediction Equations of Atmospheric Ammonia and Microclimate on Hereford Bulls Chematochemical

	Simultaneous Effect					
	Ammonia(A)-Temperature(T)		Ammonia(A)-Humidity(H)		Temperature(T)-Humidity(H)	
	Regression Equation	R^2	Regression Equation	R^2	Regression Equation	R^2
Ammonia	-	-	-	0.78	-11.53T + 0.18H	0.78
Eritrocytes	6.91-0.22A + 0.03T	0.67	8.06-0.25A-0.01H	0.78	7.59+0.04T-0.03H	0.54
Hemoglobin	12.33-0.07A-0.01T	0.41	12.23-0.02A-0.01H	0.42	15.85-0.07T-0.03H	0.76
Lymphocytes	43.08+0.02A+0.30T	0.78	58.37+0.08A-0.01H	0.68	42.37+0.31T+0.01H	0.78
Neutrophils	95.84-0.12A-0.40T	0.72	-38.98-1.67A+0.98H	0.73	38.78-1.33T+0.34H	0.74
N:L Ratio	2.65-0.04A+0.07T	0.69	-0.98-0.08+0.03H	0.64	2.19-0.05T-0.01H	0.64
% RNA rt	4.30-0.17A+0.05T	0.27	7.81-0.09A-0.03H	0.29	10.49-0.04T-0.06H	0.28
Glucose	16.85-0.67A+0.01T	0.87	18.10-0.57A-0.02H	0.88	29.82-0.06T-0.15H	0.76
Lactate	5.79-0.21A+0.01T	0.29	9.19+0.07A-0.06H	0.43	23.78-0.30T-0.13H	0.64
Hexokinase	0.01-0.01A-0.01T	0.59	0.11+0.01A-0.01H	0.63	0.07+0.01T-0.01H	0.61
PK	20.71-0.51A+0.16T	0.54	14.11-0.37A+0.02H	0.38	36.12-0.39T-0.15H	0.57

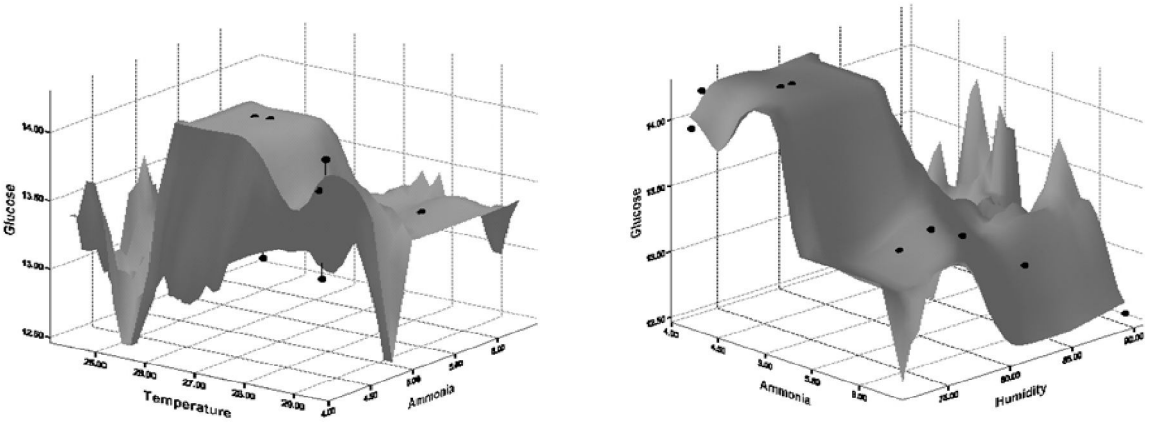

Figure 1. Relationship between Temperature, Humidity and Ammonia on Blood Glucose Concentration of Hereford Bulls

ketoglutarate to interrupt energy metabolism and ATP synthesis, so damages glucose metabolism, kexokinase and pyruvate kinase. In animals with hepatic encephalopathy, an accumulation of &-ketoglutaramate in the cerebral spinal fluid has been suggested as responsible for leading to abnormal neurotransmission. Direct experimental verification for either hypothesis is lacking. However, high energy phosphate is depleted in those regions of the brain stem, cerebellum, and cerebral hemispheres where proliferation of alzheimer type II astrocytes also occurs.

The other study by in vivo method, reported by Oba *et al*. (2005) time studies with horses showed that plasma glucose, pyruvate, and a-ketoglutarate may decline within 1 or 2 h after a toxic dose of urea is given. Thereafter these plasma constituents rise as a function of time and intensity of intoxication. Thus, differences in time of sampling with respect to onset of intoxication may explain some of the divergence of results reported by different laboratories. Hyperglycemia is refractory to exogenous insulin. The severity of the metabolic aberrations is generally a function of both degree of hyperammonemia and its duration. When animals are pretreated with urea cycle amino acids such as arginine or adapted to high protein, blood ammonia concentrations are

lower and the signs of intoxication are less severe.

This phenomenon showed that there are relationship protein metabolism between ammonia exposure. Through, result study by Regmi (2008) indicated that ammonia load does not affect the extent of glucose utilization by DMC, and that glucose carbon may not play a significant role for the synthesis of alanine, aspartate, or glutamate when DMC are exposed to increased concentrations of ammonia. But, ammonia exposure reduced metabolism commonly as effected damages olfactory system.

CONCLUSION

Negative correlation between ammonia with temperature and positive correlation between ammonia with humidity were indicated humidity plays the role on the heat and ammonia transfer. Significantly negative effect of ammonia on commonly hematochemical parameters, showed that ammonia has been reducing the function of central nervous system and metabolism. Simultaneous effect between ammonia, temperature and humidity proved to be a good indicator for predicting the profile of glucose, hexokinase, lymphocytes, neutrophyls, N:L ratio in the blood.

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