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The front cover illustrates the sketch of leaves and seeds of legume and grass forming a buffalo's horn (designed by Agung Purnomoadi)

**PREDICTION MODELS FOR OLFACTORY METABOLIC AND SOWS %RNA<sub>reticulocyte</sub>  
(RNA<sub>r1</sub>) BY MEASUREMENT OF ATMOSPHERIC AMMONIA EXPOSURE AND  
MICROCLIMATE LEVEL**

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# PREDICTION MODELS FOR OLFACTORY METABOLIC AND SOWS %RNA<sub>reticulocyt</sub> (RNA<sub>r</sub>) BY MEASUREMENT OF ATMOSPHERIC AMMONIA EXPOSURE AND MICROCLIMATE LEVEL

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## ABSTRACT

Twenty sows housed indoors in individual stalls were used to determine the relationships between atmospheric ammonia exposure and microclimate on olfactory metabolic and sows RNA<sub>reticulocyt</sub>, and to know the prediction models of the olfactory metabolic and sows RNA<sub>reticulocyt</sub> by measurement of atmospheric ammonia exposure and microclimate level. Result indicated a significantly negative effect of ammonia on commonly olfactory metabolic parameters and %RNA<sub>reticulocyt</sub>. The results also showed that ammonia has been reduced the function of olfactory receptors and activities of Ca<sup>2+</sup>-gated chloride channel open and efflux of Cl<sup>-</sup> to depolarize cell, as soon as reducing an electrical signal to the brain, so gives impact to blood metabolism (especially RNA<sub>reticulocyt</sub>). Simultaneous effect between ammonia and humidity proved to be a good indicator for predicting model of olfactory metabolic, and %RNA<sub>reticulocyt</sub> especially for creatine kinase (=16.65+0.02H-0.59A), glucose (=21.55-0.10H-0.01A), lactate (=8.87-0.03H-0.20A), ATPase (=0.05+0.00H-0.02A), adenosine triphosphate (ATP) (=13.19-0.19H+0.86A).

*Keywords: ammonia, models, olfactory, prediction, RNA<sub>r</sub>, sow*

## INTRODUCTION

The sensory cells that detect odors and tastes have much in common with the rod and cone cells. Olfactory neurons have long thin cilia extending from one end of the cell into a mucous layer that overlies the cell.

These cilia present a large surface area for interaction with olfactory signals. The receptors for olfactory stimuli are ciliary membrane proteins with the familiar G Protein-Coupled Receptor (GPCR) structure of seven transmembrane  $\alpha$  helices. Nelson and Cox (2008) reported that the olfactory signal can be any one of the many volatile compounds for which there are specific receptor proteins. Our ability to discriminate odors stems for hundreds of different olfactory receptors in the nasal passages and from the brain's ability to integrate input from different types of olfactory receptors to recognize a "hybrid" pattern.

The cilia are also the site of olfactory signal transduction. Exposure of isolated cilia from rat olfactory pithelium to numerous odorants leads to

the rapid stimulation of adenylyl cyclase and elevations in cyclic AMP (an elevation in inositol trisphosphate in response to one odorant has also been observed) (Boekhoff *et al.*, 1990, Buck and Axel, 1991). The activation of adenylyl cyclase is depended on the presence of GTP and is therefore likely to be mediated by receptor-coupled GTP-binding proteins (G proteins) (Jones and Reed, 1989). Elevations in cyclic AMP, in turn, are thought to elicit depolarization of olfactory neurons by direct activation of a cyclic nucleotide-gated, cation-permeable channel (Dhallan *et al.*, 1990). This channel is opened upon binding of cyclic nucleotides to its cytoplasmic domain, and can therefore transduce changes in intracellular levels of cyclic AMP into alterations in the membrane potential.

Exposure of ammonia continuously caused the decreasing of cyclic adenin monophosphate (cAMP) in the olfactory system, damages of olfactory signal transduction. This phenomenon is as consequence damage blood function and metabolism, especially on metabolic olfactory and hematologic.



Microclimate in the animal housing, such as temperature and humidity are important factors in animal agriculture. Temperature as the main factor can affect the health of sows and humidity plays the role on the heat transfer. Anderson *et al.* (2003) and Hongqing (2010) reported that the concentration of ammonia emissions exposure was influenced by temperature and relative humidity simultaneously. Only a limit number of investigations have been concerned about the relationship ammonia with microclimate on physiological of nasal, especially olfactory metabolic.

The objectives of this study were to explore the relationships between atmospheric ammonia exposure and microclimate on olfactory metabolic and sows RNA<sub>reticulocyt</sub> and to construct the prediction models of the olfactory metabolic and sows RNA<sub>reticulocyt</sub> based on the atmospheric ammonia exposure and microclimate level.

## 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, for 30 days during summer 2010. Twenty sows (BW average 125 ± 11.6 kg) were housed indoors in individual 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, PM) once a week for 4 weeks. Tissue sampling were conducted in the section room, at early morning (05.30, AM) every two weeks during 4 weeks study.

### Samples Analysis

**Tissue processing.** After the sows were anesthetized, the nasals were dissected from the lateral, and the anterior part of the nasal was removed by cutting 1 mm posterior to the limbus using dissection scissors and forceps. Only the nasal tissue was processed. The vitreous fluid was drained from the nasal samples prepared for biochemical assays, and the nasal tissue was carefully scraped from the eyecup. Any blood coagula or surface blood on the isolated nasal was gently removed with tweezers and absorbed in tissue paper. Nasal samples were processed in three ways: 1) they were quickly snap-frozen; 2) they were stored at -80°C until subsequent extraction of metabolites with perchloric acid; or

3) they were immediately homogenized in 0.9% saline for measurement of lactate levels, CK, or ATPase activity. Values were normalized to protein content in each sample. Alternatively, samples were immediately fixed in 4% (wt/vol) paraformaldehyde-0.01% (vol/vol) glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 30 min. After fixation, the nasals were washed in 0.1 M phosphate buffer and cryoprotected in graded sucrose solutions up to 30% (wt/vol). A cryostat (model CM3050 S, Leica, Heidelberg, Germany) was used to section the tissue in the vertical plane at a thickness of 16 µm (Cideciyan *et al.*, 2005).

**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 the first obtained. This then reacts with the surplus hydrochlorite 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. The 20 mL boric acid 0.5% was placed into adsorption bottles of each and connect 2 adsorption bottles. The ammonia gas were absorbed for 3 minutes with the flow rate of 2.0 L/min. The boric acid (sample solutions) were collected from 2 adsorption bottles, and then 10 mL sample solutions and standard solution (0, 0.2, 0.4, 0.6, and 0.8) were homogenized into the new tubes. The 5 mL solution B (containing phenol and sodium nitroprusside) and 5 mL solution C (containing NaOCl, NaOH and Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O) were added into each tubes. After one hour, absorbance of each sample was read versus blank tube by UV-1601 Spectrophotometer at 640 nm wavelength, as described by Misselbrook *et al.* (2005).

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

**Total RNA<sub>reticulocyt</sub>.** Total RNA<sub>reticulocyt</sub> were enumerated per 1000 erythrocytes in blood smears vitally stained with brilliant cresyl blue and counterstained with Wright's stain.

**Adenosine Triphosphate (ATP) and**

**phosphocreatine assays.** ATP concentration in the nasal was assayed by addition of a known amount of homogenized sample to a solution containing 50 mM Tris buffer (pH 7.4), 32 mM glucose, 0.4 mM NADP<sup>+</sup>, 12 U/ml hexokinase, 5 U/ml glucose-6-phosphate dehydrogenase, and 0.03 ml/l bovine serum albumin. The fluorescence of the reagent was measured with a fluorometer (Turner Biosystems Modulus, Sunnyvale, CA) within 30 min of sample preparation. ATP concentration in the samples was determined relative to an ATP standard curve. For the phosphocreatine assay, 1 U of CK and 0.65 mM ADP were added to each of the samples, which were then mixed by inversion. The reactions were incubated at room temperature in the dark, and the values were recorded after 60 min of incubation. The amount of ATP produced after addition of CK was subtracted from the values obtained in the first reaction. ATP and phosphocreatine values were plotted as millimoles per gram of protein.

**Creatine Kinase (CK) assay.** For determination of CK activity, left and right nasal were pooled, homogenized in 0.9% (vol/vol) saline, and briefly centrifuged at 2,800 g. The supernatant was diluted to 1:7 in saline and added to Thermo Trace CK-NAC reagent, which contains 100 mM Tris buffer, 31.5 mM creatine phosphate, 5.3 mM AMP, 2.2 mM NADP, 2.1 mM EDTA, 10.3  $\mu$ M P<sub>1</sub>P<sub>5</sub>-diadenosine 5'-pentaphosphate, 10.5 mM Mg<sup>2+</sup>, 2.7 mM ADP, 21 mM D-glucose, 21 mM N-acetyl-L-cysteine (NAC), 3,000 U/l hexokinase, and 2,000 U/l glucose-6-phosphate dehydrogenase. The change in NADH absorbance over time was measured six times for each retina by using a spectrophotometer (model UV-2501PC, Shimadzu) as described by Christie (2007). Protein content was determined using a bicinchoninic acid (BCA) assay kit. Average CK activity was calculated and is expressed in micromoles per minute per milligram of protein.

**Biochemical (lactate dehydrogenase/LDH and ATPase) assays.** LDH activity was assayed using a commercially available kit (Trace, Noble Park, Victoria, Australia), as previously described (Acosta, et al., 2005a). Total ATPase : Na<sup>+</sup>-K<sup>+</sup>-ATPase, and Mg<sup>2+</sup>-Ca<sup>2+</sup>-ATPase activities were determined on the basis of a modification of a protocol of Else (1996) as described by Acosta et al. (2005). For all assays, protein content was determined using the BCA protein assay (Acosta et al., 2005 and Doonan, 2005).

**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 (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. The 50  $\mu$ L of both samples and the lactate standards were then 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 spectrophotometer UV-1601 at 565 nm wavelength (or by microplate reader).

#### Data 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 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 metabolic olfactory parameters.

## RESULTS AND DISCUSSION

Person correlation test for the relationship of ammonia (NH<sub>3</sub>) and microclimate with olfactory metabolic and Sows RNA<sub>reticulocyt</sub> (RNA<sub>r</sub>), is presented in Table 1 and simultaneous effect and prediction models olfactory metabolic and Sows RNA<sub>r</sub> based on the atmospheric ammonia exposure and microclimate, showed in Table 2.

#### Olfactory Metabolic

Positive correlation between ammonia with humidity (Table 1) indicated that humidity played the role on the heat and ammonia transfer, proved to be an ammonia accumulation in housing. Kim et al. (2005) and Powell (2008) suggested that there were relationship between humidity and

Table 1. Pearson Correlation (r) Test for Ammonia (NH<sub>3</sub>), Microclimate, Olfactory Metabolic, and %RNA<sub>reticulocyte</sub>

	Ammonia	Temp	Humidity	%RNA <sub>ret</sub>	Glucose	Lactate	ATP	ATPase	PC	CK	LDH
Ammonia	1	-0.804	0.904	-0.546	-0.780	-0.833	0.145	-0.830	-0.638	-0.898	-0.724
Temp		1	-0.937	0.574	0.753	0.649	0.147	0.620	0.756	0.653	0.479
Humidity			1	-0.681	-0.860	-0.819	-0.177	-0.640	-0.775	-0.785	-0.607
%RNA <sub>ret</sub>				1	0.785	0.697	0.260	0.453	0.502	0.481	0.213
Glucose					1	0.892	0.199	0.513	0.588	0.802	0.377
Lactate						1	0.096	0.516	0.661	0.892	0.595
ATP							1	-0.458	0.337	-0.255	0.090
ATPase								1	0.338	0.706	0.591
PC									1	0.464	0.607
CK										1	0.589
LDH											1

RNA<sub>ret</sub> : RNA reticulocyte; TPase : Adenosine Triphosphatase; PC : Phosphocreatine; CK : Creatine Kinase; LDH: Lactate Dehydrogenase

Table 2. Simultaneous Effect and Prediction Equations of Atmospheric Ammonia and Microclimate on Sows Olfactory Metabolic, and %RNA<sub>reticulocyte</sub>

	Simultaneous Effect					
	Humidity (H) vs Ammonia (A)		Temperature (T) vs Ammonia (A)		Temperature (T) vs Humidity (H)	
	Model	R <sup>2</sup>	Model	R <sup>2</sup>	Model	R <sup>2</sup>
%RNA <sub>ret</sub>	$= 10.55 - 0.08H + 0.14A$	0.49	$= 2.89 + 0.10T - 0.09A$	0.35	$= -16.29 - 0.14T - 0.10H$	0.50
Glucose	$= 21.55 - 0.10H - 0.01A$	0.74	$= 11.74 + 0.14T - 0.27A$	0.65	$= 29.82 - 0.16T - 0.15H$	0.76
Lactate	$= 8.87 - 0.03H - 0.20A$	0.72	$= 7.90 - 0.02T - 0.35A$	0.69	$= 23.72 - 0.26T - 0.15H$	0.79
ATP	$= 13.19 - 0.19H + 0.86A$	0.78	$= -5.25 + 0.26T + 0.38A$	0.22	$= 8.85 - 0.05T - 0.04H$	0.03
ATPase	$= 0.05 + 0.00H - 0.02A$	0.75	$= 0.20 - 0.00T - 0.01A$	0.70	$= -0.17 + 0.00T - 0.00H$	0.42
PC	$= -0.11 - 0.00H + 0.00A$	0.69	$= -0.02 + 0.00T - 0.00A$	0.57	$= -0.07 + 0.00T - 0.00H$	0.61
CK	$= 16.65 + 0.02H - 0.59A$	0.81	$= 20.21 - 0.08T - 0.60A$	0.82	$= 34.75 - 0.26T - 0.17H$	0.67
LDH	$= 11.39 + 0.03H - 0.56A$	0.54	$= 17.08 - 0.12T - 0.56A$	0.55	$= -30.78 - 0.29T - 0.16H$	0.44

airborne particles, the water-carrying capacity of air as it related to temperature, and affinity of water molecules to odorous compounds.

Table 1 shows the negative correlation between ammonia with activities of olfactory metabolic (activities of Adenosine Triphosphatase/ATPase, Phosphocreatine/PC, Creatine Kinase/CK, and Lactate

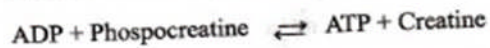
Dehydrogenase/LDH). This negative correlation indicates that ammonia caused a damages on activated olfactory receptors catalyzes guanine 5'-diphosphate-guanine 5'-triphosphate (GDP-GTP) exchange on a G protein (G<sub>olf</sub>). These phenomenon were bringing on a failure to cAMP synthesis by G<sub>olf</sub>-GTP through activates adenylyl cyclase. Hancock (2005) suggested that odorant

receptors were associated with a class of heterotrimeric G protein, here  $G_{\text{olfactory}}$ , which have their influence on adenylyl cyclase and hence cAMP signalling. The effect of ammonia emission on metabolism has been reported (van Duinkerken *et al.*, 2005, and Kalloniatis *et al.*, 2002.), and also equilibrium function damage (Starmans, 2007), olfactory nervous system and cellular metabolism (Nelson and Cox, 2008).

High level of ammonia exposure caused reducing activities rate of the adenosine triphosphate (ATP) and ATPase total. These phenomena were indicated by low glucose utilization in the olfactory organ (especially in the nasal). In Table 1, glucose showed negative correlation with ammonia that is -0.780, and in Table 2 showed that decreasing glucose effected as much as 74% by simultaneous effect of ammonia with humidity, with prediction model is  $\text{Glucose} = 21.55 - 0.1H - 0.01A$ . Glucose are precursor to form ADP/ATP (energy). Low energy in the nasal neuron caused reducing electrogenic transport by  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  has been reducing. Wang *et al.* (2003), Gasser *et al.* (2006) and Saito (2008) reported that energy was required to create and maintain an electrical potential across the neuronal plasma membrane. Yu *et al.* (2007) suggested that the membrane contains an electrogenic ATP-driven, the  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ , which simultaneously pumps  $2\text{K}^+$  ions

into and  $3\text{Na}^+$  ions out of the neuron. The greatest impact by simultaneous effect of atmospheric ammonia exposure and microclimate (Temperature and humidity) clearly defined by damages nasal tissue, by phospho creatine and creatinekinase parameters.

The enzyme creatine kinase catalyzes the reversible reaction (Nelson and Cox, 2008):



Depletion of ADP and glucose like described at previous paragraph, not only decreased catalyzes rate of creatine kinase, but also decreased the production of ATP. Therefore, when ammonia exposure level increase caused creatine kinase (CK) decreased (significantly negative correlation = -0.898, in Table 1), in Table 2 and Figure 1, showed that decreasing CK effected as much as 81% by simultaneous effect of ammonia vs humidity, while ammonia vs temperature and temperature vs humidity were affected 82% and 67% respectively, with prediction model were  $\text{CK} = 21.55 - 0.1H - 0.01A$ ;  $\text{CK} = 20.21 - 0.08T - 0.60A$ ; and  $\text{CK} = 34.75 - 0.26T - 0.17H$ , respectively.

$\% \text{RNA}_{\text{reticulocyte}} (\% \text{RNA}_{\text{t}})$

Action potentials are the chief mechanism of the information transfer in the nervous system, so depletion of ATP (as impact of low glucose) in

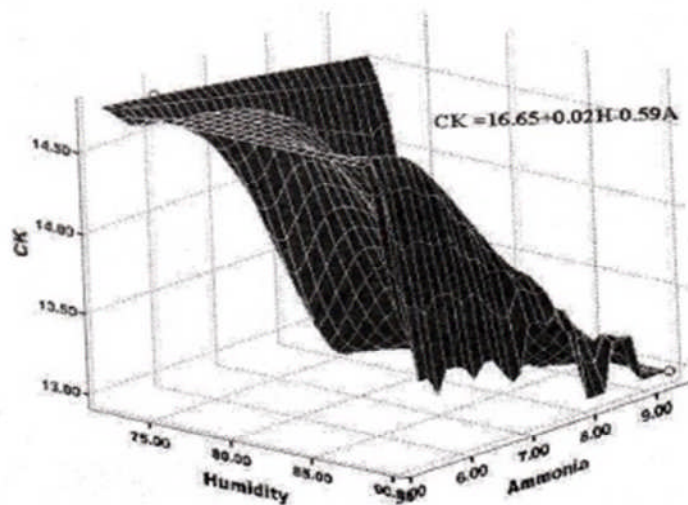


Figure 1. Relationship between humidity and ammonia on creatinekinase (CK) concentration of Sows



neuron would have disastrous effects on all activities coordinative by neuron signal, included the activities of  $Ca^{2+}$ -gated chloride channel open and efflux of  $Cl^-$  to depolarizes the cell and triggering an electrical signal to the brain (Gomperts *et al.*, 2009, Yamasaki *et al.*, 2005). Condition low triggering an electrical signal to the brain caused %RNA<sub>n</sub> very low (Zong *et al.*, 2004) (negative correlation = -0.546 in Table 1), and in Table 2 showed that decreasing %RNA<sub>n</sub> effected 49% by simultaneous effect of ammonia with humidity, with prediction model was %RNA<sub>n</sub> = 10.55 - 0.08H - 0.14A.

### CONCLUSION

Significantly negative effect of ammonia on commonly olfactory metabolic parameters and %RNA<sub>reticulocyt</sub>, showed that ammonia reduced the function of olfactory receptors and activities of  $Ca^{2+}$ -gated chloride channel open and efflux of  $Cl^-$  to depolarizes the cell, and soon as reducing an electrical signal to the brain, so it gives impact to blood metabolism (specially RNA<sub>n</sub>). Simultaneous effect between ammonia and humidity proved to be a good indicator for predicting model of olfactory metabolic, and %RNA<sub>n</sub> specially for creatine kinase, glucose, lactate, ATPase and Adenosine triphosphate (ATP).

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