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Stress and antioxidative status of Zovawk piglets at different intervals of weaning

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Zovawk is an indigenous pig breed of India whose home tract is in different parts of Mizoram. This pig is of scavenging type and are reared by Mizo community in rural areas of Mizoram. As a part of All India Co-ordinated Research Project (AICRP) on Pigs, the Zovawk pigs were brought from different parts of Mizoram and are being reared in College of Veterinary Science and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram.

In rural farming conditions, there is no practice of scientific weaning of Zovawk piglets. As a part of routine farm practice, the Zovawk piglets are weaned from their mothers on 56th day of age. In different pig breeds, weaning has been indicated to be stressful (Yin *et al.* 2014) that contributes to intestinal and immune system dysfunctions (Campbell *et al.* 2013), low antioxidative status (Yin *et al.* 2014). Many health status parameters have been detected to alter in response to weaning at different intervals (Zhu *et al.* 2013, Escribano *et al.* 2019, Yu *et al.* 2019).

Zovawk pigs have been reported to possess blood and biochemical parameters resembling to both the wild and domestic pigs (Mayengbam *et al.* 2014). Previous study indicated adaptive characteristic of Zovawk to domestication (Mayengbam and Tolenkhomba 2017). There is, however, no literature available to indicate the stress and antioxidative status of Zovawk piglets in response to weaning. The present study was therefore carried out in order to know the impact of weaning on the stress and antioxidative status of Zovawk piglets.

The present study was carried out on Zovawk piglets reared in the AICRP on Pig, College of Veterinary Science and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram. The average body weight of Zovawk piglets on the day of weaning was 3.3 kg. Weaning was done on 56th day of age.

Blood samples were collected from 5 weaned piglets of 1 litter at three intervals *i.e.* 0, 7th and 14th day of weaning. Blood samples were collected by puncturing the anterior venacava of each piglet by using 24 Gauze needle. EDTA coated blood collection vials were used for collection of blood. Blood smears were prepared from fresh blood samples. Differential leukocyte count (DLC) was conducted on blood smears of each sample by the method of Jain (1986). N:L was calculated from the result of DLC.

The collected blood samples were centrifuged at 3000 rpm for 20 min to separate plasma. Plasma was separated and harvested in fresh tubes. The packed cells left after separation of plasma were washed 3 times by centrifugation with equal volume of normal saline solution. The supernatant along with the buffy coat was discarded after every wash. To prepare 1% hemolysate, 100 µl of washed RBC was mixed with 9.9 ml of 0.05 M phosphate buffer saline, pH 7.4.

Protein concentration of plasma and hemolysate was estimated by Biuret method (Lubran 1978). Concentration of cortisol, thiobarbituric acid reactive substances (TBARS), ferric reducing antioxidant power (FRAP) were estimated in plasma while superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and reduced glutathione (GSH) were estimated in hemolysate. Cortisol was estimated by using enzyme linked immunosorbent assay kit (Elabscience Biotechnologies, Texas, United States). Activity of SOD was estimated by nitro blue tetrazolium reduction method described by Nishikimi (1972). GPx activity was estimated by the measure of rate of oxidation of glutathione by H_2O_2 as described by Rotruck *et al.* (1973). Catalase activity was determined by method of Aebi (1984). GSH was estimated by following the procedure of Griffith (1980). FRAP was estimated by the method of Benzie and Strain (1999).

Saliva samples were collected at 6 intervals i.e. 0, 3rd, 6th, 9th, 12th, and 15th day of weaning. Sampling was done twice a day i.e. half an hour before and after feeding in the morning. Sponge was used to soak the saliva. Saliva-soaked sponge was put in a syringe and saliva was separated from sponge by pushing the syringe. The collected saliva was centrifuged at 2500 rpm for 15 min. The supernatant was transferred into a fresh tube and used for estimation of salivary alpha amylase (sAA) activity. sAA activity was estimated by using diagnostic test strips in automatic blood analyser, Fuzifilm DRI-CHEM 4000i.

Statistical analyses were done using the protocol reported by Snedecor and Cochran (1994). SPSS version

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16 was used to analyze effect of weaning on different parameters in the present study.

There was significant increase in plasma cortisol ($P<0.05$) of Zovawk on 14th day of weaning (Table 1). The increase in plasma cortisol could be due to weaning stress. Zhu *et al.* (2013) and Yu *et al.* (2019) reported that weaning stress significantly increased the plasma cortisol level. Weaning along with mixing of piglets after weaning was found to cause agonistic behaviour in piglets that was found to associate with significant increase in plasma cortisol (Merlot *et al.* 2004). Salivary cortisol was also found to increase after 1 and 2 days of weaning in piglets (Escribano *et al*. 2019). Slow increase in level of plasma cortisol of Zovawk could be due to late weaning age. The plasma cortisol level of Zovawk piglets estimated in the current study was observed to be in higher range (Table 1) as compared to previous reports of Koopmans *et al*. (2006) in crossbred piglets (Landrace × Yorkshire). This variation could be due to difference in breed characteristics and their adaptation to different climatic condition.

The N:L of Zovawk decreased significantly ($P<0.01$) on 7th day of weaning which further decreased significantly on 14th day of weaning (Table 1). White blood cell count has been found to increase significantly from day 0 to 4th day and 11th day of weaning in piglets (Sugiharto *et al.* 2014). In controlled situations, an increase in neutrophil lymphocyte ratio was one indicator of the pig's response to stress (Widowski *et al.* 1989). N:L was one of the indexes of stress and adaptability of animals to the social rank (Hjarvard *et al.* 2009) and local environment which generally increased under the stress condition (Minka and Ayo 2007). Decrease in lymphocyte and eosinophils, and increase in neutrophil occurred in pigs under stress such as following marketing (Clemens *et al.* 1986) and electric shock (Maeda *et al.* 2011). N:L ratio was found to increase in piglets as a response to cold stress (Nienaber *et al.* 1989) and weaning stress (Davis *et al.* 2004). Gardner *et al.* (2001) observed increase in N:L on 3rd day of weaning and decrease on 10th day of weaning. The present finding of decrease in N:L on 7th day of weaning and further decrease on 14th day of weaning could be due to the pattern of fall of N:L after 3rd day of weaning (Gardner *et al.* 2001). N:L of Zovawk pigs was within the range reported earlier by Mayengbam (2020). N:L of Zovawk was slightly higher than those reported in Nicobari pig and Andaman desi pig (De *et al.* 2013) and lower than those reported in Large White Yorkshire (Aladi *et al.* 2008).

Weaning also decreased the activity of SOD (Table 1). SOD activity remained stable till 7th day of weaning and declined on 14th day of weaning ($P<0.01$). SOD activity was also found to decrease after weaning in other piglets (Zhu *et al.* 2012). The down regulation of antioxidant enzymes after weaning had also been reported in piglets with decrease in SOD content in serum (Zhu *et al.* 2013). Chedea *et al*. (2018) found decrease in SOD activity in colon of weaned piglets. Present finding of decrease in SOD activity along with the previous reports indicated that weaning stress caused decline in SOD activity. Tao *et al*. (2016) reported that SOD activity continuously decreased from day 1 to day 7 post weaning. Leng *et al*. (2010) reported significant decrease in SOD activity after 7 days of weaning and early weaning age caused more severe stress than the late weaning age. Slow decline in SOD activity of Zovawk piglets in the present study could be the result of late weaning age. The SOD activity of Zovawk piglets estimated in the present study was within the range recorded in previous study of Zhu *et al.* (2012).

The GPx activity, catalase activity, GSH and FRAP concentration showed no significant change after weaning. There was however declining trend in GPx activity and GSH after weaning (Table 1). Decrease in GPx activity has been indicated in weaning stress (Leng *et al*. 2010, Zhu *et al.* 2013, Yin *et al*. 2014, Luo *et al*. 2016, Chedea *et al*. 2018). The GPx activity of Zovawk was in higher ranges as compared to the previous reports (Zhu *et al.* 2012). There was a wide individual variation in GPx and catalase activities and GSH concentration in the present study.

Table 1. Effect of weaning on stress and antioxidative biomarkers of Zovawk piglets

Parameter	Day of weaning			F value
	0	7th	14th	
Cortisol (ng/mL)	64.41^{a} ±3.14	67.29^{a} ±4.61	80.64^{b} ±3.35	4.33*
N:L	0.50^{c} ±0.01	0.43^{b} ±0.01	0.39^{a} ±0.01	21.58**
SOD (U/mg protein)	25.66^{b} ±2.13	20.88^{b} ±1.53	14.53^{a} ±2.17	8.07**
GPx (U/mg protein)	247.22 ±39.25	181.03 ±26.98	132.08 ±29.78	3.17^{NS}
Catalase (U/mg protein)	357.25 ±32.58	336.85 ±77.75	339.57 ±135.60	0.01^{NS}
GSH (U/mg protein)	84.54 ±19.92	50.95 ±11.89	33.63 ±6.91	3.45^{NS}
TBARS (µM/L)	492.17^{c} ±102.39	295.18^{ab} ±50.26	98.54^{a} ±30.24	8.35**
FRAP (mM/L)	0.26 ±0.06	0.30 ±0.06	0.29 ±0.05	0.15^{NS}

Means with different superscripts in the same row differ significantly. ^{NS}Indicates non-significant difference between the days of weaning.

Concentration of TBARS of Zovawk piglets in the present study decreased significantly on 7th day of weaning and remained high on 14th day of weaning (Table 1). The decline in TBARS could be due to weaning stress in piglets as weaned group of pigs had significantly reduced concentration of TBARS than the piglets which remained with the mother (Wei *et al*. 2017). The level of plasma TBARS of Zovawk was observed to be in higher range as compared to previous reports in other pigs (Papadopoulos *et al.* 2016).

Activity of sAA has been reported to be an indicator of emotional and physiological stress (Granger *et al.*

2007, DeCaro 2008, Nater and Rohleder 2009). Martínez-Miró *et al.* (2016) suggested sAA as an indicator of sympathetic, adrenal medullary and hypothalamic pituitary adrenocortical system. In the present study, Zovawk piglets were found to decrease the sAA activity significantly on 3rd day of weaning which further decreased on 9th day of weaning (Table 2). From 9th day onwards, sAA activity remained stable till 15th day of weaning. Hudman *et al.* (1957) reported that sAA activity increased after 7 days of weaning that was followed by decrease in activity when the piglets were 35 days of age irrespective of weaning or suckling the mother. Pierzynowska *et al.* (2018) reported that plasma amylase activity decreased with age. They recorded decrease in activity of plasma amylase activity by 50% after 2 months of weaning when the weaning was done at 4 weeks of age. The present finding of decrease in sAA activity could be the result of age but not the result of weaning as the Zovawk piglets were 56 days old that was older age as compared to previous studies (Hudman *et al.* 1957, Pierzynowska *et al.* 2018).

Table 2. sAA activity of Zovawk at different intervals of weaning

Day of weaning						F value
0	3rd	6th	9th	12th	15th	
4592.00^{c} ±1014.91	2989.50^{b} ±631.79	1629.50^{ab} ±358.81	640.00^{a} ±101.77	500.50^{a} ±77.78	386.50^{a} ±65.62	10.84**

Means with different superscripts differ significantly.

Weaning caused stress in Zovawk piglets. Weaning stress was detected by presence of higher cortisol on 14th day of weaning in Zovawk. Activity of sAA of Zovawk was found to associate with age which decreased gradually after weaning in Zovawk. Weaning stress led to fall in antioxidative status of Zovawk piglets with decrease in activity of SOD and concentration of TBARS.

SUMMARY

Stress and antioxidative status of weaned Zovawk piglets were analysed by estimating stress and antioxidative biomarkers. Cortisol, neutrophil to lymphocyte ratio (N:L), SOD, GPx, catalase and concentration of GSH, TBARS and FRAP were estimated in blood samples on 0, 7th and 14th day of weaning. sAA activity was estimated in saliva samples on 0, 3rd, 6th, 9th, 12th and 15th day of weaning. Weaning stress caused changes in antioxidative status. On 14th day of weaning, cortisol concentration increased significantly while SOD and TBARS decreased significantly. N:L decreased on 7th and 14th day of weaning. GSH showed a declining trend in response to weaning while the catalase and FRAP were not influenced by weaning. sAA decreased significantly on 3rd day of weaning that decreased further on 9th day of weaning.

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REFERENCES

Aebi H. 1984. Catalase *in vitro*. *Methods in Enzymology* **105**: 121-26.

Aladi N O, Okeudo N J, Okoli I C and Akanno E C. 2008. Reproductive and hematological characteristics of the Nigerian indigenious and Large White Yorkshire pigs in humid tropical environment. *Asian Journal of Animal and Veterinary Advances* **3**(1): 17–23.

Benzie I F and Strain J J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* **299**: 15–27.

Campbell J M, Crenshaw J D and Polo J. 2013. The biological stress of early weaned piglets. *Journal of Animal Science and Biotechnology* **4**(1): 1–4.

Chedea V S, Palade L M, Marin D E, Pelmus R S, Habeanu M, Rotar M C, Gras M A, Pistol G C and Taranu I. 2018. Intestinal absorption and antioxidant activity of grape pomacepolyphenols. *Nutrients* **10**(5): 588.

Clemens E T, Schultz B D, Brumm M C and Jesse G W. 1986. Influence of market stress and protein level on feeder pig hematologic and blood chemical values. *American Journal of Veterinary Research* **47**: 359–62.

Davis M E, Maxwell C V, Erf G F, Brown D C and Wistuba T J. 2004. Dietary supplementation with phosphorylated mannans improves growth response and modulates immune function of weanling pigs. *Journal of Animal Science* **82**(6): 1882–91.

De A K, Kundu A, Kundu M S, Sunder J and Jeyakumar S. 2013. Comparative study on haematological traits of endangered Andaman wild pig and other indigenous pig breeds available at Andaman and Nicobar Islands, India. *Veterinary World* **6**(10): 794–98.

DeCaro J A. 2008. Methodological considerations in the use of salivary α-amylase as a stress marker in field research. *American Journal of Human Biology* **20**(5): 617–19.

Escribano D, Ko H L, Chong Q, Llonch L, Manteca X and Llonch P. 2019. Salivary biomarkers to monitor stress due to aggression after weaning in piglets. *Research in Veterinary Science* **123:** 178–83.

Gardner J M, Duncan I J H and Widowski T M. 2001. Effects of social "stressors" on belly-nosing behaviour in early-weaned piglets: is belly-nosing an indicator of stress*? Applied Animal Behaviour Science* **74**(2): 135–52.

Granger D A, Kivlighan K T, el-Sheikh M, Gordis E B and Stroud L R. 2007. Salivary alpha-amylase in biobehavioral research: recent developments and applications. *Annals of the New York Academy of Sciences* **1098**(1): 122–44.

Griffith O W. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinyl pyridine. *Analytical Biochemistry* **106**: 207–12.

Hjarvard B D, Larsen O N, Juui-Madsen H R, Jorgensen E and Jensen K H. 2009. Social rank influences the distribution of blood leukocyte subsets in female growing pigs. *Scandinavian Journal of Laboratory Animal Sciences* **36**(4): 309–20.

Hudman D B, Friend D W, Hartman P A, Ashton G C and Catron D V. 1957. Digestive enzymes of the baby pig. Pancreatic and salivary amylase. *Journal of Agriculture and Food Chemistry* **5**(9): 691–93.

Jain N C. 1986. *Schalm's Veterinary Hematology*. 4th edn. WB

Saunders and Company, Philadelphia, Lea and Febiger. pp. 1221.

Koopmans S J, Guzik A C, Van Der Meulen J, Dekker R, Kogut J, Kerr B J and Southern L L. 2006. Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets. *Journal of Animal Science* **84**(4): 963–71.

Leng J and Zhu R J. 2010. Effects of weaning stress at different ages on the serum biochemical and immune indices of piglets *Journal of Anhui Agricultural Sciences* **38**(4): 1853–54. (Article in Chinese)

Lubran M M. 1978. The measurement of total serum proteins by the Biuret method. *Annals of Clinical and Laboratory Science* **8**(2): 106–10.

Luo Z, Zhu W, Guo Q, Luo W, Zhang J, Xu W and Xu J. 2016. Weaning induced hepatic oxidative stress, apoptosis, and aminotransferases through MAPK signaling pathways in piglets. *Oxidative Medicine and Cellular Longevity*. http://dx.doi.org/10.1155/2016/4768541

Maeda Y, Tomioka M, Hanada M and Oikawa M. 2011. Changes in peripheral blood lymphocyte and neutrophil counts and function following long term road transport in thoroughbred horses. *International Journal of Applied Research in Veterinary Medicine* **9**(3): 284–89.

Martínez-Miró S, Tecles F, Ramón M, Escribano D, Hernández F, Madrid J, Orengo J, Martínez-Subiela S, Manteca X and Cerón J J. 2016. Causes, consequences and biomarkers of stress in swine: an update. *BMC Veterinary Research* **12**(1): 1–9.

Mayengbam P and Tolenkhomba T C. 2017. Effect of sex on hematological profile of Zovawk and indigenous pig of Mizoram hills. *Indian Journal of Hill Farming* (Special Issue): 100–05.

Mayengbam P, Tolenkhomba T C and Ali M A. 2014. Hematological profile of Zovawk and indigenous pig of Mizoram. *Veterinary World* **7**(7): 505–08.

Mayengbam P. 2020. Hematobiochemical Profile of Indigenous Pig 'Zovawk' of Mizoram. Scholar"s Press, 17 Meldrum Street, Beau Bassin 71504, Mauritius.

Merlot E, Meunier-Salaün M C and Prunier A. 2004. Behavioural, endocrine and immune consequences of mixing in weaned piglets. *Applied Animal Behaviour Science* **85**(3-4): 247–57.

Minka N S and Ayo J O. 2007. Physiological responses of transported goats treated with ascorbic acid during the hot dry season. *Animal Science Journal* **78**(2): 164–72.

Nater U M and Rohleder N. 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology* **34**(4): 486–96.

Nienaber J A and Hahn G L. 1989. Cool nighttime temperature and weaning age effects on 3 to 10 week old pigs. *Transactions of the ASAE* **32**(2): 691–95.

Nishikimi M, Rao N A and Yagi K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical Biophysical Research Communication* **46**(2): 849–54.

Papadopoulos G A, Kanoulas V, Arsenos G, Janssens G P, Buyse J, Tzika E D and Fortomaris P D. 2016. Effects of palygorskite dietary supplementation on back fat mobilization, leptin levels and oxidative stress parameters in sows. *Applied Clay Science* **132**: 535–41.

Pierzynowska K G, Lozinska L, Woliński J and Pierzynowski S. 2018. The inverse relationship between blood amylase and insulin levels in pigs during development, bariatric surgery, and intravenous infusion of amylase. *PLoS OnE* **13**(6): e0198672.

Rotruck J T, Pope A L, Ganther H E, Swanson A B, Hafeman D G and Hoekstra W. 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science* **179**(4073): 588–90.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th edn. East-West Press Pvt. Ltd., New Delhi.

Sugiharto S, Hedemann M S and Lauridsen C. 2014. Plasma metabolomic profiles and immune responses of piglets after weaning and challenge with *E. coli*. *Journal of Animal Science Biotechnology* **5**: 17.

Tao X, Xu Z and Men X. 2016. Transient effects of weaning on the health of newly weaning piglets. *Czech Journal of Animal Science* **61**(2): 82–90.

Wei H K, Xue H X, Zhou Z X and Peng J. 2017. A carvacrol–thymol blend decreased intestinal oxidative stress and influenced selected microbes without changing the messenger RNA levels of tight junction proteins in jejunal mucosa of weaning piglets. *Animal* **11**(2): 193–201.

Widowski T M, Curtis S E and Gravesc N. 1989. The neutrophil:lymphocyte ratio in pigs fed cortisol. *Canadian Journal of Animal Science* **69**: 501–04.

Yin J, Wu M M, Xiao H, Ren W K, Duan J L, Yang G and Yin Y L. 2014. Development of an antioxidant system after early weaning in piglets. *Journal of Animal Science* **92**(2): 612–19.

Yu C H, Chen C Y and Chang C C. 2019. The immediate effects of weaning stress on the hypothalamus-pituitary-adrenal alteration of newly weaned piglets. *Journal of Animal Physiology and Animal Nutrition* **103**(4): 1218–23.

Zhu L, Cai X, Guo Q, Chen X, Zhu S and Xu J. 2013. Effect of N-acetyl cysteine on enterocyte apoptosis and intracellular signaling pathways' response to oxidative stress in weaned piglets. *British Journal of Nutrition* **110**(11): 1938–47.

Zhu L H, Zhao K L, Chen X L and Xu J X. 2012. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. *Journal of Animal Science* **90**(8): 2581–89.