Contributed paper

# GENETIC PARAMETERS FOR URINALYSIS TRAITS RECORDED ON GESTATING SOWS

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#### SUMMARY

Urinalysis can be used to detect sows that typically remain unidentified with health conditions such as urinary tract infection, and also provides data on physiological variables reflecting metabolic status (e.g. glucose, ketones). The urine was collected from gilts and sows (N=694) after animals were transferred to the farrowing shed. The traits were defined from the urinalysis test strip results, with additional subjective measurements of odour, colour and turbidity. Subsequently, a trait representing urinary tract infection was defined. Heritability estimates were in a range 0.08 to 0.36, except for the presence of blood (0.03). Strong genetic correlations were estimated between bilirubin and urobilinogen (0.78), but not other trait combinations. The study demonstrated that several urinalysis traits could be considered as selection criteria for increasing the health status of sows. However, alternative procedures to collect phenotypes are required to improve ease of data collection. The associations of urinalysis parameters with breeding objective traits requires further investigation.

## INTRODUCTION

Undetected and untreated urinary tract infection (UTI), high ketones (demonstrating metabolic disorders) or low Vitamin C (potentially demonstrating nutritional deficiency) result in poor performance and increased sow removals (Almond 2005; Theil *et al.* 2013; Nielsen *et al.* 2019). Mazutti *et al.* (2013) proposed using reagent strips to detect UTI routinely, enabling treatment. In this study, we estimated genetic parameters for urinalysis variables recorded with reagent strips using urine samples from late gestation sows. To the knowledge of the authors, no previous study reported parameters for similar traits. Our hypothesis was that variation in health and metabolic state has a genetic basis, such that variables obtained from urinalysis are heritable, potentially providing opportunities to enhance health status of sows from both production and genetic perspectives.

#### MATERIALS AND METHODS

**Data.** Urinalysis data was recorded at two independent nucleus farms over 5 weeks, between October-November 2017 (Farm A, N = 254 sows), and 8 weeks between March-May 2017 (Farm B, N = 440 sows) according to kit instructions (CombiScreen®VET 11 PLUS). Urine was collected once per sow, on average 5 days before farrowing, in the early morning before the first feeding event. The urinalysis test strips evaluated levels of bilirubin, urobilinogen, ketones, Vitamin C, glucose, protein, blood, pH, nitrite, leucocytes, and specific gravity according to CombiScreen®VET 11 PLUS (Table 1). Each variable was scored in levels representing concentrations. Urine colour was subjectively scored on a scale of 1 to 3 (pale, normal, dark), while odour and turbidity were scored as absent (0) or present (1). The presence of a urinary tract infection (UTI) was inferred as absent (0) or present (1) if nitrite was positive and pH  $\geq$  6. Levels for urinalysis parameters are shown in Table 1.

<sup>\*</sup> A joint venture of NSW Department of Primary Industries and the University of New England

**Analyses.** Data preparation was carried out using R (R Core Team 2020). During the process of data preparation, where the description of levels on test strip was expressed in characters, observed values were replaced with numeric values (e.g. nitrite + or ++ to 1 or 2). The square root transformation was performed for ketones, glucose, protein, blood and leucocytes, and for specific gravity x 100 (recorded in 0.005 increments), due to the non-normal distribution of data for these traits. Variances are presented on the transformed scale.

Data from both farms were combined to estimate genetic parameters. Sows were progeny of 283 sires and 553 dams. The pedigree was extended back by 5 generations to a total of 1261 sires and 3274 dams. Estimates of variance components were obtained by fitting a linear mixed animal model using residual maximum likelihood procedures in ASReml (Gilmour *et al.* 2014). Systematic effects were parity group (4 levels: parities 1, 2, 3-4, >4) and selection line nested within farm (10 levels). Genetic ( $r_A$ ) and phenotypic ( $r_P$ ) correlations were estimated using a series of bivariate analyses. Both genetic and phenotypic correlations were reported only for traits where heritability was above 0.05, due to the large standard errors, which would result in unreliable estimates for genetic correlations.

#### **RESULTS AND DISCUSSION**

Heritability estimates. Bilirubin and urobilinogen, typically indicators of liver disease, were moderately heritable,  $0.33 \pm 0.13$  and  $0.34 \pm 0.14$  (Table 1).

Trait	Units	Levels	Normal values	Normal (%)	$h^2 \pm SE$	$\sigma^2_{\ p}$	R <sup>2</sup> (%)
Bili	mg/dl	0,1,2,4	0	60.5	$0.33\pm0.13$	1.20	2.84
Uro	mg/dl	0,2,4	0	78.2	$0.34\pm0.14$	0.61	20.6
Ket*	mg/dl	0,10,25,100	0	96.1	$0.10\pm0.11$	0.96	0.46
Vit C	mg/dl	0,1,2	0	24.5	$0.16\pm0.10$	0.45	3.60
Glucose*	mg/dl	0,2,5,14,28	0	92.1	NE		
Protein*	mg/dl	0,15,30,100,500	0	27.3	$0.20\pm0.13$	11.5	8.16
Blood*	Ery/ml	0,10,50,300	0	80.5	$0.03\pm0.09$	6.95	1.28
pH§		5.0-8.5	5.5-8.0	48.9	$0.11\pm0.10$	0.44	0.26
Nitrite	µmol/l	0,1,2	0	93.9	$0.36\pm0.13$	0.19	0.00
Leuco*	Leuco/µl	0,25,75,500	0	95.0	NE		
$\operatorname{Spec} G^{\dagger}$		1-1.03	1.02-1.04	54.7	$0.33\pm0.13$	1.00	1.77
UTI	0/1	0,1	0	95.4	$0.20\pm0.12$	0.04	0.00
Odour	0/1	0,1	0	74.8	$0.32\pm0.13$	0.17	10.8
Colour		1-3	1-3		$0.14\pm0.10$	0.38	2.04
Turb	0/1	0,1	0	73.1	$0.08\pm0.10$	0.15	0.60

Table 1: Urinalysis variables with recording levels, normal values and percentage of sows, heritability estimates ( $h^2$ ), phenotypic variance ( $\sigma^2_p$ ) and the coefficient of determination ( $R^2$ )

Notes: \* square root transformation applied; § in 0.5 increments; Bili: bilirubin; Uro: urobilinogen; Ket: ketones; Vit C: Vitamin C; Leuco: leucocytes; SpecG † specific gravity in 0.005 increments (×100); Turb: turbidity; NE: not estimable

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The heritability estimate for ketones was  $0.10 \pm 0.11$ . An increased level of ketones in urine is an indicator of ketosis or diabetes mellitus. Although ketosis in sows is not well investigated, it could affect feed intake during lactation, and consequently weight and fat loss during the late gestation and lactation (Alsop *et al.* 1994). Urine colour and odour were lowly to moderately heritable ( $0.14 \pm 0.10$  and  $0.32 \pm 0.13$ ). These two traits are strongly dependent on the concentration of urine. In contrast, heritability for turbidity was low,  $0.08 \pm 0.10$ . Turbidity may indicate presence of contaminants, such as blood, bacteria, epithelia, cells or crystals, implying non-genetic variation. Similarly, the presence of blood in urine was not heritable. Heritabilities were not estimable for glucose and leucocytes due to the low number of positive animals for these measurements in one of the farms. The failure to detect urinary glucose pre-farrowing might reflect the practice of restricted feeding during gestation and pre-farrowing. The heritability for UTI inferred from nitrite and pH levels was  $0.20 \pm 0.12$ . This trait is considered as a predisposing factor for reproductive disorders, MMA (mastitis-metritis-agalactia), and lower milk production (Petersen 1983). Therefore, genetic variation in predisposition to developing UTI might be related to genetic variation in health and reproductive performance of sows.

**Correlations.** The genetic correlation  $(r_A)$  between bilirubin and urobilinogen was strong (0.78  $\pm$  0.19, Table 2) and both are indicators of liver disease.

	Bili	Uro	Ket	Vit C	Protein	pН	Nitrite	SpecG	UTI	Odour	Colour	Turb
Bili		0.78	0.07	0.99	0.85	-0.07	0.25	0.48	-0.04	0.35	0.29	0.43
		(0.19)	(0.51)	(0.20)	(0.17)	(0.44)	(0.28)	(0.23)	(0.36)	(0.26)	(0.35)	(0.50)
Uro	0.46		-0.17	0.45	0.74	0.21	0.21	0.28	-0.20	0.46	0.20	0.34
	(0.03)		(0.48)	(0.31)	(0.27)	(0.46)	(0.28)	(0.28)	(0.37)	(0.27)	(0.39)	(0.49)
Ket	-0.04	-0.01		-0.27	-0.06	0.81	-0.99	-0.37	-0.64	-0.97	-0.30	-0.38
	(0.04)	(0.04)		(0.53)	(0.60)	(0.53)	(0.63)	(0.39)	(0.55)	(0.62)	(0.54)	(0.88)
Vit C	0.36	0.22	0.00		0.60	0.60	0.01	0.47	-0.68	0.29	0.94	0.62
	(0.04)	(0.04)	(0.04)		(0.31)	(0.57)	(0.36)	(0.28)	(0.47)	(0.34)	(0.32)	(0.56)
Protein	0.55	0.32	0.01	0.31		-0.16	-0.13	0.22	-0.26	0.22	0.40	0.73
	(0.03)	(0.04)	(0.04)	(0.04)		(0.56)	(0.35)	(0.34)	(0.44)	(0.36)	(0.42)	(0.58)
pН	0.15	0.12	-0.04	-0.12	0.05		0.78	-0.39	0.14	0.16	0.17	-0.08
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)		(0.58)	(0.39)	(0.51)	(0.45)	(0.58)	(0.80)
Nitrite	0.11	0.20	0.02	0.02	0.16	0.01		-0.51	0.96	1.00	-0.36	0.79
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)		(0.30)	(0.19)	(0.31)	(0.40)	(0.47)
SpecG	0.38	0.17	0.03	0.46	0.33	-0.35	-0.03		-0.86	0.27	0.78	0.29
	(0.04)	(0.04)	(0.04)	(0.03)	(0.04)	(0.04)	(0.04)		(0.41)	(0.27)	(0.23)	(0.47)
UTI	0.06	0.09	-0.01	-0.02	0.12	0.22	0.63	-0.12		0.91	-0.58	0.92
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.02)	(0.04)		(0.37)	(0.54)	(0.62)
Odour	0.30	0.18	-0.04	0.10	0.25	0.07	0.29	0.18	0.23		0.12	0.70
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)		(0.39)	(0.39)
Colour	0.42	0.21	-0.04	0.40	0.35	-0.01	-0.03	0.59	-0.02	0.21		0.05
	(0.03)	(0.04)	(0.04)	(0.03)	(0.04)	(0.04)	(0.04)	(0.03)	(0.04)	(0.04)		(0.67)
Turb	0.21	0.22	-0.07	0.03	0.19	0.18	0.27	0.06	0.22	0.53	0.12	
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.03)	(0.04)	

Table 2: Genetic (above diagonal) and phenotypic correlations for urinalysis traits with standard errors in subscript

Bili: bilirubin; Uro: urobilinogen; Ket: ketones; Vit C: Vitamin C; SpecG: specific gravity; Turb: turbidity. Bold values are significantly different from zero. Phenotypic correlations ( $r_P$ ) amongst several urinalysis traits suggest that values were not fully independent of each other. This could be because of correlated errors due solely to the test strip chemistry, or because urinalysis variables were correlated with each other from a physiological perspective. High Vitamin C levels can result in a false positives for bilirubin and nitrite (CombiScreen®VET 11 PLUS). Therefore, it is unclear whether strong  $r_A$  (0.99 ± 0.20) and moderate  $r_P$  (0.36 ± 0.04) correlations between Vitamin C and bilirubin were due to this effect. Phenotypic correlations were moderate to high between specific parameters and the traits that were conditional on their values (e.g. UTI and nitrite or pH levels). Scored variables (colour, odour, turbidity) were positively associated with each other and also many urinalysis parameters, indicating abnormal levels, but are not diagnostic of particular conditions. Strong, positive correlations were found between odour and nitrite and/or UTI, suggesting that strong urine odour could potentially be used as a trigger to initiate testing for the presence of an infection and treatment where required.

#### CONCLUSIONS

Urinalysis provides an opportunity to obtain data related to health and metabolic status of sows, which has utility from both management and genetic perspectives, and can be considered as selection criteria for breeding programs. However, associations with other selection criteria or breeding objective traits are required, along with proof that selection would generate meaningful changes in the health status of sows. Phenotypically, test strips are a useful tool to identify and treat unwell sows. However, collecting urine samples is laborious and better strategies need to be developed for routine recording.

## ACKNOWLEDGEMENT

This research was funded by the Australasian Pork Research Institute Ltd. (project 2A-116). The first author was supported by UNE via International Postgraduate Research Award (UNE IPRA).

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