EXOGENOUS OESTROGEN: EFFECT OF OESTRADIOL BENZOATE ON BLOOD CHEMISTRY OF LARGE WHITE PIGS

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ABSTRACT

The effect of oestradiol benzoate (OB) on the haematology and serum chemistry of large white pigs in a tropical environment was investigated. Forty-eight pigs (24 males and 24 females) were administered oestradiol benzoate at 0.2mg/kg live weight or 1.0 ml corn oil (control group), once weekly for 24 weeks, from birth. Blood sampling was done at birth, 5 weeks (weaning), 10 weeks, 20 weeks and 24 weeks respectively. The haematological and serum biochemical analyses were carried out.The haematological parameters analyzed include: packed cell Volume (PCV); Haemoglobin (HB); Red blood cell count (RBC); White Blood cell count (WBC); while Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration were derived. The Serum biochemical parameters of interest include: Total serum protein, Albumin, Cholesterol, Alanine Transaminase, Glucose. The results showed that for Packed cell Volume (PCV) of oestradiol groups had significantly higher values ($P<0.05$) and sex effect was significant in favour of females. This same trend was observed for haemoglobin concentration except that sex effect was not significant. Red cell and white cell counts followed similar trends. For serum chemistry, sex effect was not significant at the periods blood was sampled. But oestradiol benzoate significantly increased serum albumin, glucose and total protein but resulted in lower serum total cholesterol. Results suggest that oestradiol benzoate could be administered to pigs for improved production without any adverse effect on the health status of the pigs as indicated by the blood chemistry.

Key Words: Oestradiol benzoate, pigs, serum.

INTRODUCTION

Swine production represents an important segment in the food animal industry throughout the world (Cromwell, 1991). The consumption of food of animal origin increases with increase in the human population. Part of the human population is profitably engaged in swine production as a source of income. Today's modern pork is much leaner than it was in the past and is widely accepted by consumers. Most of the pigs in Nigeria are raised in Southern and the Middle Belt regions. Pigs in these areas are raised in a variety of ways, from outdoor lots to complete confinement. Swine farms vary in size from small to very large, with some of the large farms having thousands of sows. Regardless of the size of the operation or the condition under which pigs are produced, there is the need to properly define the effect of oestrogen (oestradiol benzoate) on pigs from day old to puberty and also to evaluate how it can be used to improve swine production in the tropics especially as literature are conflicting on its effect in animal production. Oestradiol is the major oestrogen secreted by the ovary. Oestrogens are anabolic steroids similar but weaker than testosterone. It is synthesized in the Graaffian follicle, corpus luteum and placenta from cholesterol with testosterone as an intermediate (Tripatthi, 1999).Oestrogens bring about pubertal changes: growth of uterus, fallopian tubes and vagina. It

also accelerates development of secondary sex characters. They are important in maintaining bone mass and promoting positive calcium balance. Oestradiol esters injected intramuscularly are slowly absorbed and exert prolonged action (Ganong, 1997). In circulation, oestradiol are largely plasma protein bound to sex steroid-binding globulin as well as to albumin. Oestradiol is rapidly oxidized in the liver to estrone, which is hydroxylated to form estriol. Estriol thus derived from estrone is conjugated with glucoronic acid and sulphate which are excreted in urine and bile. Oestrogens are used mainly as contraceptives and for hormone replacement therapy. They are also useful in therapy of atrophic vaginitis, delayed puberty, dysmenorrhea, hirsutism, carcinoma prostate and suppression of Lactation. Furthermore, oestrogens have been indicated in sexual precocity (Ganong, 1997). This experiment was set up to determine the effect of exogenous oestrogen (oestradiol) on pigs from day-old to 24 weeks of age with a view to evaluating it for use in improving swine production in the tropics.

MATERIALS AND METHODS

The experiment was carried out at the Piggery Unit of the Teaching and Research Farm, University of Ibadan, Ibadan.

A total of 48 Large White pigs consisting of 24 males and 24 females were used. Half the number of each sex served as controls with no oestradiol benzoate injection. At birth the piglets were weighed and randomly divided into control and treatment groups. Identification was by use of permanent colour markers to inscribe treatment or experimental codes and numbers, the hormone injections were obtained from local veterinary pharmacy shops in Ibadan. The injections were given by deep intra-muscular injections in thigh muscles of the hind-legs. The dosage per pig was 0.2mg oestradiol benzoate (equivalent to 0.16mg oestradiol, Shanghai pharmaceutical company, China) per kilogram body weight. The animals were injected between the hours of 7 and 8am once weekly from day-old to 24 weeks of age. The treatments are designated CF for control female with only corn oil injection, and EF for female with Oestradiol Benzoate injection. Blood sampling was carried out at birth (0 week), 5 weeks (early weaning age); 10 weeks (normal weaning age, which corresponds to half the gestation time), 20 weeks (pre-pubertal age) and 24 weeks (peri-pubertal and pubertal age depending on sex). Blood collected at the stated periods were analyzed for full haematology; the serum harvested was analyzed for total protein, Albumin, glucose, urea, aspartate transaminase, alkaline phosphatase, acid phosphatase, cholesterol, creatinine, bilirubin and calcium using appropriate reagent kits, readings were taken using a spectrophotometer at the recommended wave length for each parameter. The statistical model for this experiment was:

$$Y_{ij} + \mu + \beta_i + T_j + \epsilon_{ij}$$

where:

Y_{ij} = individual observation for the j^{th} treatment in the i^{th} block

μ = general mean

β_i = effect of the i^{th} block (sex is the blocked factor)

T_j = effect of the j^{th} treatment (Oestradiol injection)

ϵ_{ij} = experimental error

Each experimental treatment consisted of 3 replicates of 2 animals each. Data were analyzed by 2-way ANOVA (complete randomized block design) of SAS, 2001 and significant means were separated using the Duncan multiple range test of the same software.

RESULTS

Table 1 shows the packed cell volume (PCV) of pigs administered oestradiol and the controls. The PCV value for the control female (CF) was 37.80+0.86 and that for Oestrogen (EF) injected was higher (though not significant $P>0.05$) at 47.50$\pm$0.7. At week 10, the PCV values for CF had increased, so also were the PCV values for EF. There was a further increase in the subsequent weeks 20 and 24 for the CF; however, there was no significant change in PCV value for EF. For the males, Control Male(CM) had PCV value of 37.00+0.86 and EM had 46.92$\pm$0.26. There were significant differences ($P<0.05$) in PCV values at weaning for both CM and EM, at week ten, there was an increase in the PCV values obtained in the CM whereas, in the EM there was no significant difference: The PCV values dropped at week 20 in the CM while it increased in the EM. At week 24, the PCV values for control increased while it decreased in the EM. All through the weeks, there

were significant differences (P<0.05) in the PCV value for CM and EM. The PCV obtained for EF was 47.509+0.77 at weaning while that of EM was 46.92+0.26. The PCV values for EF increased in subsequent weeks except for week 24, however, there was not significant increase in the PCV values for EM as from week 10 to week 20, it differs significantly in week 20 and at week 24 where it dropped, from weaning to the 20th week, there was significant difference for both sexes except at week 24 where there were no significant differences (P>0.05) for both sexes.

Table 2 shows the Haemoglobin (Hb) concentration of pigs administered Oestradiol and the controls.

The haemoglobin at weaning for CF and EF differed significantly (P<0.05). In CF it was increased in week 10, decreased in week 20 and subsequently increased. Whereas in EF,Haemoglobin (HB) only increased in 10th and remains the same until week 24. There was however significant difference (P<0.05) in the Hb levels in both CF and EF.In the males, there were significant difference in the Hb values obtained in CM and EM it was observed that, there were significant increase from weaning to week 10, seen in both CM and EM, while it decreased in CM in week 20 and later increased in week 24, it only decreased at week 24 in the EM in both sexes EF and EM shows no significant difference in their Hb levels.

Table 3 shows the RBC count for pigs injected with oestradiol.

The RBC values for female, CF and EF differ significantly. The values of RBC in the EF decrease up to the 24th week while in the CF the values increased in week 20 unto the 24th week. The RBC values in the males, for CM at weaning was 6.65- 0.55 for the EM, there were significant difference in both CM and EM at weaning and all through the experiment. In week 10, there was drop in the RBC values in both CM and EM, increased in week 20, CM but continued to decrease throughout the experiment in EM In both sexes, there were significant differences in the RBC values. For EF and EM the values decreased from weaning to the end of the experiment.

Table 4 show the white blood cell count (WBC) of pigs injected with Oestradiol benzoate

The WBC values obtained in females, CF at weaning is significantly different to that obtained in EF, and this difference is sustained throughout the duration of the experiment. In the CF it increased up to the 20th week and dropped at week 24, the same trend is observed in the WBC values in EF. In Males the CM and EM WBC values differs significantly (P<0.05). There was consistent increase to week 20 and a decrease in WBC values of CM in 24th week, whereas, in EM the WBC values decreased in week 10, increased in week 20 and decreased in week 24.

There were significant differences in the WBC values of in both sexes for EF and EM. The trend observed in WBC values for EF differs with that observed in EM. In the EF the WBC values increased from 10th week, decreased in 20th week and also increased in 24th week.

Table 8 shows the mean values of some serum biochemical parameters of pigs injected with Oestradiol Benzoate. The parameters included glucose, Total protein, Albumin, Albumin globulin ratio, cholesterol and Aspartate amino transaminase (AST). At weaning (5 weeks) oestradiol injected pigs had significantly higher glucose concentration than control pigs, this higher serum glucose concentration was observed for oestradiol injected pigs for week 10, 20 and 24. At weaning, oestradiol injected pigs had significantly lower serum total protein than its controls. The reverse was observed at 10 weeks and 24 weeks, while at 20 weeks serum total protein concentrations were similar. Albumin globulin ratio also followed the trend observed for serum total protein. Oestradiol injected pigs were observed to have significantly lower serum total cholesterol during the sampling periods of the experiment. For AST, controls had lower serum AST concentrations at weaning, but higher values at 10 weeks and 24 weeks and similar values to injected pigs at 20 weeks. Serum concentrations of Bilirubin, Calcium, Urea, ACP, ALP and Creatinine were determined only at the 24th week of experiment. There were no significant differences in serum concentrations of these metabolites between the control and the treated groups.

TABLE: 1 Effect of oestradiol benzoate on packed cell volume (%)in pigs

SEX	TREATMENT	WEANING (5)	WEEKS 10	20	24
	C	37.00 ± 0.86^{b}	40.25 ± 0.96^{b}	37.33 ± 0.47^{b}	40.67 ± 1.13^{b}
F	E	47.50 ± 0.77^{a}	48.00 ± 0.73^{a}	48.50 ± 0.88^{c}	48.50 ± 0.58^{a}
	C	37.00 ± 0.86^{b}	40.25 ± 0.95^{b}	37.33 ± 0.47^{b}	40.67 ± 1.13^{b}
M	E	46.92 ± 0.26^{a}	46.92 ± 0.47^{a}	49.08 ± 0.26^{c}	48.33 ± 0.40^{a}
BOTH	EF	47.50 ± 0.77^{a}	47.00 ± 0.73	47.50 ± 0.58	48.50 ± 0.58
SEXES EM		46.92 ± 0.26	46.92 ± 0.42	49.08 ± 0.26	48.33
0.40					

abc: means in the same sex column differently superscripted differ significantly

TABLE 2: Effect of oestradiol benzoate onhaemoglobin concentration (g/dl)in pigs

SEX	TREATMENT	WEANING(5)	WEEKS 10	20	24
	C	12.38 ± 0.36^{b}	13.46 ± 0.36^{b}	12.46 ± 0.16	13.60 ± 0.38^{b}
F	E	15.88 ± 0.26^{a}	16.05 ± 0.20^{a}	16.23 ± 0.20	16.23 ± 0.20^{a}
	C	12.38 ± 0.36^{b}	13.46 ± 0.32^{b}	12.46 ± 0.16^{b}	13.60 ± 0.38^{b}
M	E	15.66 ± 0.09	15.65 ± 0.16^{a}	16.40 ± 0.09^{c}	16.25 ± 0.13^{a}
BOTH	EF	15.80 ± 0.26	16.05 ± 0.25	16.23 ± 0.20	16.23 ± 0.20
SEXES EM		15.66 ± 0.09	15.65 ± 0.16	16.40 ± 0.09	16.25 ± 0.13

abc: means in the same column within each sex group with different superscripts are significantly different ($P < 0.05$).

TABLE 3: Effect of oestradiol benzoate onred blood cell count ($10^{6}/\mu l$)in pigs

SEX	TREATMENT	WEANING (5)	WEEKS 10	20	24
	C	6.65 ± 0.22^{a}	6.30 ± 0.24^{a}	8.08 ± 0.40^{a}	5.85 ± 0.07^{a}
F	E	5.61 ± 0.23^{b}	5.13 ± 0.14^{b}	5.07 ± 0.09^{b}	5.07 ± 0.09^{a}
	C	6.65 ± 0.22^{a}	6.30 ± 0.24^{a}	8.08 ± 0.40^{a}	5.85 ± 0.07^{a}
M	E	6.22 ± 0.25	5.83 ± 0.10	5.59 ± 0.09^{b}	5.81 ± 0.09^{b}
BOTH	EF	5.61 ± 0.23^{a}	5.13 ± 0.14^{b}	5.07 ± 0.09^{b}	5.07 ± 0.09^{b}
SEXES EM		6.22 ± 0.25	5.83 ± 0.10^{a}	5.59 ± 0.09	5.51 ± 0.09^{a}

abc: means in the same column within each sex group with different superscripts are significantly different ($P < 0.05$).

TABLE 4: Effect of oestradiol benzoate on White Blood Cell count ($10^{3}/\mu l$) in pigs

SEX	TREATMENT	WEANING (5)	WEEKS 10	20	24
	C	10.26 ± 0.09^{b}	10.53 ± 1.05^{b}	15.75 ± 1.44	10.25 ± 0.71^{b}
F	E	22.80 ± 0.70^{a}	24.34 ± 0.58^{a}	19.58 ± 2.55	23.40 ± 0.70^{a}
	C	10.26 ± 0.90^{b}	10.53 ± 1.05^{b}	15.75 ± 1.44	10.25 ± 0.71^{b}
M	E	25.88 ± 0.31^{a}	24.63 ± 0.49^{a}	25.40 ± 0.22^{c}	24.03 ± 0.41^{a}
BOTH	EF	22.80 ± 0.70^{b}	24.33 ± 0.58	19.58 ± 2.55^{b}	23.40 ± 0.70
SEXES EM		25.88 ± 0.31^{a}	24.63 ± 0.49	25.40 ± 0.22^{a}	24.03 ± 0.41

abc: means in the same column within each sex group with different superscripts are significantly different ($P < 0.05$).

TABLE 5: Effect of Oestradiol Benzoate on Mean Corpuscular Volume (fl) of Pigs

		WEEKS			
SEX	TREATMENT	WEANING (5)	10	20	24
	C	56.01 ± 1.22^{b}	64.52 ± 1.99^{b}	47.19 ± 1.89	69.46 ± 1.25^{b}
F	E	85.60 ± 2.29^{a}	94.24 ± 2.43^{a}	95.76 ± 1.10	95.70 ± 0.83^{a}
		56.01 ± 1.22^{b}	64.52 ± 1.99^{b}	47.19 ± 1.89	69.46 ± 1.25^{b}
M	E	76.44 ± 2.33	80.63 ± 1.10	87.93 ± 1.13^{a}	87.90 ± 0.71^{c}
BOTH	EF	85.60 ± 0.29^{a}	94.24 ± 2.43^{a}	95.76 ± 1.10^{a}	95.70 ± 0.88^{a}
SEXES	EM	76.44 ± 0.33^{b}	80.63 ± 1.10^{b}	87.93 ± 1.12^{b}	87.90 ± 0.21^{b}

abc: means in the same column within each sex group with different superscripts are significantly different ($P < 0.05$).

TABLE 6: Effect of Oestradiol Benzoate on Mean Corpuscular Haemoglobin (pg) of pigs

		WEEKS			
SEX	TREATMENT	WEANING (5)	10	20	24
	C	18.70 ± 0.38^{b}	21.57 ± 0.66^{b}	15.75 ± 0.63^{b}	23.22 ± 0.43^{b}
F	E	28.62 ± 0.76^{a}	31.50 ± 0.81^{a}	32.04 ± 0.37^{a}	32.02 ± 0.30^{a}
	C	18.70 ± 1.38^{b}	21.57 ± 0.66^{b}	15.73 ± 0.63^{b}	23.22 ± 0.43^{b}
M	E	25.52 ± 0.78^{a}	26.90 ± 0.37^{a}	29.38 ± 0.37^{a}	29.87 ± 0.33^{a}
BOTH	EF	28.62 ± 0.76^{a}	31.50 ± 0.89^{a}	32.04 ± 0.37^{a}	32.02 ± 0.30^{a}
SEXES	EM	25.52 ± 0.78^{b}	28.90 ± 0.37^{b}	29.38 ± 0.37^{b}	29.57 ± 0.33^{b}

abc: means in the same column within each sex group with different superscripts are significantly different ($P < 0.05$).

TABLE 7: Effect of Oestradiol Benzoate on Mean Corpuscular Haemoglobin Concentration (%) of Pigs

		WEEKS			
SEX	TREATMENT	WEANING (5)	10	20	24
	C	33.40 ± 0.30	33.40 ± 0.00	33.40 ± 0.03	33.40 ± 0.00
F	E	33.50 ± 0.00	33.40 ± 0.00	33.50 ± 0.00	33.50 ± 0.00
	C	33.40 ± 0.30	33.40 ± 0.00	33.30 ± 0.00^{b}	33.40 ± 0.00
M	E	33.40 ± 0.00	33.40 ± 0.00	33.40 ± 0.00^{a}	33.70 ± 0.20
BOTH	EF	33.50 ± 0.00	33.40 ± 0.00	33.50 ± 0.00^{a}	33.50 ± 0.00
SEXES	EM	33.40 ± 0.00	33.40 ± 0.00	33.40 ± 0.00^{b}	33.70 ± 0.20

abc: means in the same column within each sex group with different superscripts are significantly different ($P < 0.05$).

TABLE: 8 Effect of Oestradiol Benzoate on Serum Chemistry of Pigs

TREATEMENT	PARAMETER	WEEKS			
		WEANING (5)	10	20	24
C	GLUCOSE , mg/dl	72.50 ± 2.42	89.17 ± 3.98^{b}	115.00 ± 5.15	120.00 ± 3.26^{b}
P	GLUCOSE , mg/dl	104.17 ± 1.49	114.17 ± 1.49^{a}	125.83 ± 2.38	131.66 ± 1.67^{a}
C	TOTAL PROTEIN	5.32 ± 0.04^{a}	5.70 ± 0.03^{b}	6.31 ± 0.09	6.80 ± 0.03^{b}
P	TOTAL PROTEIN	5.19 ± 0.03^{b}	5.85 ± 0.04^{a}	6.23 ± 0.05	7.02 ± 0.05^{a}
C	ALBUMIN, g/dl	2.52 ± 0.01^{a}	2.55 ± 0.01^{b}	2.93 ± 0.02	3.32 ± 0.01^{b}
P	ALBUMIN, g/dl	2.46 ± 0.01^{a}	2.72 ± 0.01^{a}	2.88 ± 0.01^{a}	3.47 ± 0.01^{a}
C	ALB/GLO RATIO	0.90 ± 0.00	0.81 ± 0.00^{b}	0.86 ± 0.01	0.95 ± 0.01
P	ALB/GLO RATIO	0.90 ± 0.00	0.87 ± 0.01^{a}	0.86 ± 0.01	0.96 ± 0.01
C	CHOLESTEROL, mg/dl	100.09 ± 0.42	121.17 ± 0.39^{a}	131.42 ± 0.40^{a}	142.08 ± 0.43^{a}
P	CHOLESTEROL, mg/dl	46.67 ± 1.12^{b}	50.42 ± 0.74^{b}	52.92 ± 0.74^{b}	61.25 ± 0.65^{b}
C	AST (GOT), i.u/l	22.50 ± 0.34^{b}	22.50 ± 0.36^{a}	21.50 ± 0.36	21.00 ± 0.25^{a}
P	AST (GOT)	24.75 ± 0.35^{a}	21.50 ± 0.26^{b}	20.75 ± 0.25	19.92 ± 0.19^{b}
C	CREATININE, mg/dl	-	-	-	1.85 ± 0.01
P	CREATININE, mg/dl	-	-	-	1.80 ± 0.01
C	UREA, mg/dl	-	-	-	16.65 ± 0.40
P	UREA	-	-	-	16.00 ± 0.30

abc: means in the same column within each treatment/parameter group with different superscripts are significantly different
($P < 0.05$)

Table 9: Blood profile of piglets at birth

Parameters	PCV (%)	Hb (g/dl)	RBC ^{6}/µl)	WBC (10^3/µl)	MCV (fl)	MCH (pg)	MCHC (%)	Glucose (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin/ Globulin ratio	Cholesterol (g/dl)	AST
Sex														
Male	30.25±1.37	9.81±0.23	4.39±0.35	11.48±1.10	69.24±2.01	22.43±0.45	32.40 0.30	± 102.08±3.73	2.47±0.15	0.36 0.15	± 2.11 0.04	± 0.17±0.01	68.20±2.89	19.00±0.86
female	30.92±1.18	10.03±0.48	4.26±0.18	9.65±0.74	72.89±1.75	23.60±0.41	32.42 1.19	± 98.33±3.37	2.48±0.15	0.40 0.02	± 2.07 0.04	± 0.19±0.00	65.33±4.82	19.42±0.75

abc: Means in the same row within each sex group with different superscript are significantly different ($P<0.05$).

PCV = Packed cell volume
Hb = Haemoglobin concentration
Rbc = Red blood cell count
Wbc = White blood cell count (total)
MCV = Mean Corpuscular volume
MCH = Mean Corpuscular Haemoglobin
MCHC = Mean Corpuscular Haemoglobin Concentration.
AST = Aspartate amino transaminase.

DISCUSSION

The result of Serum biochemistry in this study agrees with the report of Tripalthi (1999) and Ganong (1997) who reported that oestrogen decrease blood cholesterol. Blood sugar is slightly elevated. They further stated that blood coagulability is increased due to induction of synthesis of clotting factors. The result of the aforementioned authorsagrees with the results on Haematology in this experiment.Exogenous oestradiol resulted in serum hormones being elevated to pubertal levels at weaning this suggests that it can indicates in a write constitutional precocity as observed by also by Ganong (1997).Adejumo and Egbunike, (1989) have demonstrated that age, sex, rate of growth, feeding intervals and animals' immediate behaviour at the time of sampling exert some effects on carbohydrate, protein and lipid metabolic parameters.The creatinine level indicates the extent of muscle wastage and subsequent degradation of muscle phosphor-creatinine to form creatinine. Doornenbal *et al.* (1986) reported that creatinine originated almost entirely (98%) in striated muscles. Creatinine can be used as an indirect measure of protein quality (Eggum, 1970). It has been supplied as an index of lean tissue mass in the body. Doornenbal *et al.* (1986) reported an average creatinine value of 95.0 $\pm$ 11.7µmole/litre for growing pigs and that pigs fed *ad libitum* had significantly lower level of creatinine compared to pigs with restricted feed. Exogenous hormone had significant ($P < 0.05$) effect on the serum cholesterol levels of pigs between the groups. The observed values were in agreement with the normal range of 67 – 174mg/dl reported by Mitruka and Rawnsley (1977).In addition to serum total proteins, urea can also be used for indirect measurement of index for evaluating protein quality (Eggum, 1970). Urea is the main nitrogenous end- product arising from the catabolism of amino acids that are not used in the biosynthetic reactions in mammals. The result of this work showed a non-significant variation in serum urea concentrations among treatment groups. This is in agreement with the findings of Wilson *et al.* (1972) who found that the level of blood urea in monogastrics does not remain constant. However, Doornenbal *et al.* (1983) reported increase in serum urea with increasing age.

CONCLUSION

It can be concluded from this study that oestradiol benzoate significantly influenced the blood chemistry of pigs. Sex effect was significant.

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