INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

BEHAVIOURAL AND PHYSIOLOGICAL EFFECTS OF 915 MHz MICROWAVE RADIATION ON WEANER PIGS

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Master of Science

in the Department of Anatomy and Physiology

Faculty of Veterinary Medicine

University of Prince Edward Island

Kimberley D. Foote

Charlottetown, P. E. I.

November, 1999

CONDITIONS OF USE OF THE THESIS

SIGNATURE PAGES

iii-iv

REMOVED

SUMMARY

Microwave radiation (915 MHz) was investigated as a form of supplemental heat for early weaned pigs. Infrared lamps were used as the industry control. Behavioural parameters including aggressive behaviour and activity level as well as average daily gain (ADG) were used as an indication of piglet health and welfare upon exposure to microwaves. Microwave exposure was found to cause a power level-dependent decrease in activity level ($P < 0.05$) in weaner pigs which diminished over a three week period of exposure. Average daily gain was not significantly affected ($P > 0.05$) by microwave exposure. No evidence of heat stress or discomfort was displayed by pigs in either treatment. Daily percent resting time was positively correlated ($P < 0.05$) with microwave power level, room temperature and skin temperature.

A subsequent study was carried out to investigate the basis of the microwave induced depression of activity. Serial blood samples were taken over a 24 hr period and plasma cortisol, glucose and melatonin levels were determined. Microwave radiation was found to induce a power level-dependent increase ($P < 0.05$) in plasma melatonin and glucose concentrations. However, plasma cortisol concentrations were similar for microwave and infrared-exposed pigs ($P > 0.05$) indicating that microwaves were not causing stress-induced changes in behaviour or plasma melatonin and glucose.

ACKNOWLEDGEMENTS

I would like to thank Maritime Electric, Atlantic Canada Opportunities Agency, Industrial Research Assistance Program, Department of the National Research Council, and D'Ossone Canada, Ltd. for their assistance in this research project. I would also like to thank the members of the following departments: audio visual, anaesthesia repairs, animal resources, and computer services, as well as the laboratory technicians and Dr. Ortenburger. I would especially like to thank all my friends and my supervisor, Dr. Bate, without whom this thesis would not have been possible.

TABLE OF CONTENTS

1.0 LITERATURE REVIEW 1

1.1 Introduction 1

1.2 Problem of early weaning 4
- 1.2.1 Dietary change 5
- 1.2.2 Piglet immunity 6
- 1.2.3 Effect of early weaning on subsequent litter size 7
- 1.2.4 Space and temperature requirements 8
- 1.2.5 Social stress 9
- 1.2.6 Effect of early weaning on growth 9

1.3 Climatic physiology 10
- 1.3.1 Effect of environmental conditions on temperature requirements of pigs. . 12
- 1.3.2 Thermoregulatory mechanisms 12
- 1.3.3 Methods of heat loss from the pig 13
- 1.3.4 Effect of inadequate environmental temperature on growth 14

1.4 Behaviour patterns of domestic pigs 15
- 1.4.1 Social behaviour 16
- 1.4.2 Activity patterns 17
- 1.4.3 Feeding and drinking behaviour 18

1.5 Characteristics of mammalian sleep 18
- 1.5.1 Definition of sleep 18
- 1.5.2 Sleep structure 19
- 1.5.3 Sleep physiology 20
  - 1.5.3.1 Sleep centers 20
  - 1.5.3.2 Sleep inducing agents 22
- 1.5.4 Control of the sleep-wake cycle 24
- 1.5.5 Regulation of melatonin synthesis and metabolism 25
- 1.5.6 Relationship between melatonin, sleep and thermoregulation 27
- 1.5.7 Melatonin secretion in pigs 30
- 1.5.8 Melatonin and electromagnetic waves 31

1.6 Microwave radiation 32
- 1.6.1 Generalities 32
- 1.6.2 Characteristics of electromagnetic waves 33
  - 1.6.2.1 Frequency 33
  - 1.6.2.2 Period 33
  - 1.6.2.3 Wavelength 33

1.6.2.4 Impedence 34
1.6.2.5 Power 34
1.6.2.6 Phase 35
1.6.3 Microwave propagation 35
1.6.4 Reflection 36
1.6.5 Attenuation 38
1.6.6 Absorption 39

1.7 Biological effects of electromagnetic radiation 39
1.7.1 Introduction 39
1.7.2 Behavioural effects of microwave radiation 40
1.7.3 Thermal vs. athermal effects of Mw radiation 44

1.8 Objectives of the research 49

2.0 ACTIVITY LEVEL AND PERFORMANCE OF WEANER PIGS EXPOSED TO 915 MHz MICROWAVE RADIATION 50

2.1 Summary 50

2.2 Introduction 51

2.3 Materials and methods 52
2.3.1 Treatments 52
2.3.2 Microwave equipment 52
2.3.3 Animals 54
2.3.4 Environmental conditions 55
2.3.5 Video equipment 56
2.3.6 Behavioural observations 56
2.3.7 Temperature measurements 57
2.3.8 Statistical analyses 57

2.4 Results 58
2.4.1 Activity level 58
2.4.2 Performance 61
2.4.3 Correlations 61

2.5 Discussion 66

2.6 Conclusions 71

3.0 PLASMA MELATONIN, CORTISOL, AND GLUCOSE CONCENTRATIONS IN WEANER PIGS EXPOSED TO 915 MHz MICROWAVE RADIATION .. 72

3.1 Summary 72

3.2 Introduction 73

3.3 Materials and methods 76
3.3.1 Experimental design 76
3.3.2 Equipment 77
3.3.3 Environmental conditions 78
3.3.4 Cannulation surgery 78
3.3.5 Radiotransmitter implantation 81
3.3.6 Blood collection 81
3.3.7 Biochemical analyses 82
3.3.8 Behavioural observations 84
3.3.9 Statistical analyses 84

3.4 Results 85
3.4.1 Melatonin data 85
3.4.2 Cortisol data 93
3.4.3 Glucose data 96
3.4.4 Correlations 99

3.5 Discussion 101

3.6 Conclusion 110

4.0 GENERAL DISCUSSION 112

5.0 APPENDIX A 120

6.0 REFERENCES 122

LIST OF TABLES

2.0 ACTIVITY LEVEL AND PERFORMANCE OF WEANER PIGS EXPOSED TO 915 MHz MICROWAVE RADIATION

Table I. Wilk's Lambda *P* values for pretreatment and experimental analyses of variance testing pattern differences in daily percent resting time 59

Table II. Average body weights and standard deviations per treatment for weaner pigs exposed to IR, Mw at 11.4 mW cm^{-2} (Mw1) or Mw at 6.1 mW cm^{-2} (Mw2) (N = 32) . 62

Table III. Pearson Correlation Coefficients and *P* values for comparisons between resting time, power level, room temperature, cage temperature, skin temperature and relative humidity 63

3.0 PLASMA MELATONIN, CORTISOL, AND GLUCOSE CONCENTRATIONS IN WEANER PIGS EXPOSED TO 915 MHz MICROWAVE RADIATION

Table I. *P* values for pretreatment and experimental analyses of variance testing differences in mean plasma melatonin concentrations (pg mL^{-1}) 86

Table II. *P* values for repeated measures analysis of variance testing differences in mean plasma concentration of melatonin (pg mL^{-1}), cortisol (μg dL^{-1}) and glucose (mg dL^{-1}) . 87

Table III. *P* values for pretreatment and experimental analyses of variance testing differences in mean plasma cortisol concentrations (μg dL^{-1}) 94

Table IV. *P* values for pretreatment and experimental analyses of variance testing differences in mean plasma glucose concentrations (mg dL^{-1}) 97

Table V. Pearson correlation coefficients and *P* values for comparisons between pig body temperature, microwave power level, humidity level, room temperature, plasma melatonin and percent sleep time 100

LIST OF FIGURES

2.0 ACTIVITY LEVEL AND PERFORMANCE OF WEANER PIGS EXPOSED TO 915 MHz MICROWAVE RADIATION

Figure 1. Diagramatic representation of the set up for microwave and infrared heated cages setup 53

Figure 2. Mean percentage resting times per day for IR-vs. Mw-treated pigs (at two Mw power levels). Arrow indicates beginning of treatments, and data points represent average over 24h period.. 60

Figure 3. Effect of Mw power level (W) (x) on daily percentage resting time ($Y = 79.1 + 0.03\ x$; $r^2 = 0.22$) 64

Figure 4. The effect of daily average skin temperature (°C) (x) on daily percentage resting time ($Y = 56.8 + 0.73\ x$; $r^2 = 0.03$) (N = 156) 65

3.0 PLASMA MELATONIN, CORTISOL, AND GLUCOSE CONCENTRATIONS IN WEANER PIGS EXPOSED TO 915 MHz MICROWAVE RADIATION

Figure 1. Mean plasma melatonin concentrations (pg mL^{-1}) ± SEM for IR, Mw1, and Mw2 with outliers deleted. The shaded area represents the dark phase of the sampling period. Data points represent the average of all pigs sampled per treatment 89

Figure 2. Mean plasma melatonin concentrations (pg mL^{-1}) ± SEM for replicate 1 (IR, Mw1 and Mw2) with outliers deleted. Shaded areas represent the dark phase of the sampling period 91

Figure 3.Mean plasma melatonin concentrations (pg mL^{-1}) ± SEM for replicate 2 (IR, Mw1 and Mw2) with outliers deleted 92

Figure 4. Mean cortisol concentrations (µg dL^{-1}) ± SEM for IR (n=10 for 800-1330 then n=9), Mw1 (n=6) and Mw2 (n=6) treatment groups ... 95

Figure 5. Glucose concentrations (mg dL^{-1}) ± SEM for IR (n=9 for 800-1330 then n=8), Mw1 (n=6) and Mw2 (n=6) groups 98

5.0 APPENDIX A

Figure 1. Body core temperatures (°C) during the pretreatment (time = -3,-2, and -1 days before treatment) and treatment (time = 0.5 - 23.5 h) periods for replicates 1 (Mw2) (3.8 mW cm-2) (n = 1) and 2 (Mw2)

GLOSSARY OF ABBREVIATIONS

ADG	Average daily gain
ANSI	American National Standards Institute
ARAS	Ascending reticular activating system
BBB	Blood brain barrier
BW	Body weight
CNS	Central nervous system
CT	Cage temperature
C.V.	Coefficient of variation
CW	Continuous wave
DRL	Differential reinforcement of low rate
EEG	Electroencephalogram
EET	Effective environmental temperature
EHF	Extremely high frequency
ELF	Extremely low frequency
EM	Electromagnetic
F	Frequency
FR	Multiple schedule fixed ratio
GH	Growth hormone
GHz	Gigahertz
GRF	Growth hormone releasing factor
5-HIAA	5-Hydroxyindoleacetic acid

HIOMT	Hydroxy-indole O-methyltransferase
HPA	Hypothalamo-pituitary-adrenal
5-HT	5-Hydroxytryptamine (serotonin)
Hz	Hertz
IM	Intramuscular
IRPA	International Radiational Protection Association
IR	Infrared radiation
IU	International units
IV	Intravenous
λ	Wavelength
LCT	Lower critical temperature
lx	Lux
ME	Metabolizable energy
MHz	Megahertz
Mw	Microwave
NAT	N-acetyl transferase
PCPA	Parachlorophenylalanine
PGO	Ponto-geniculo-occipital
POAH	Preoptic-anterior hypothalamus
PS	Paradoxical sleep
PW	Pulsed wave
ρ	Reflection coefficient

ρ^2	Power reflection coefficient
REM	Rapid eye movement
RH	Relative humidity
RT	Room temperature
SAR	Specific absorption rate
SCN	Suprachiasmatic nucleus
SHF	Super high frequency
ST	Skin temperature
SWR	Standing wave ratio
SWS	Slow wave sleep
TE	Transverse electric
TEM	Transverse electric and magnetic
TM	Transverse magnetic
TSH	Thyroid stimulating hormone
UCT	Upper critical temperature
UHF	Ultra high frequency
μT	Microtesla
V	Volts
VLF	Very low frequency
W	Watts

1.0 LITERATURE REVIEW

1.1 Introduction

The main goal of any animal production system is to maximize production at the least cost to the producer while maintaining a suitable end product that will meet consumer demands and, at the same time, maintain an adequate standard of animal welfare. The swine industry has advanced tremendously over the past 30 years toward maximizing production and improving pork. Techniques such as genetic selection for desirable traits (Berruecos et al., 1970; Cleveland et al., 1982; Cameron and Curran, 1994), improved health management and nutrition (Wilson and Leibholz, 1981; Whang and Easter, 1995) and an overall better understanding of pig physiology and behaviour have made these advancements possible. Weaning piglets at an early age has increased pig production. Wilson et al. (1986) studied the lactation length of Ontario swine herds between 1979-1981 and found that the average was 37.4 $\pm$ 8.3 days. A shorter lactation length of 27.6 $\pm$ 7.5 days was reported by Dewey et al. (1994) for Ontario swine herds within the period 1987-1991. In the study by Dewey et al. (1994), lactation length ranged from as little as 4 days to as long as 40 days. Early weaning shortens the farrowing interval of the sow thereby increasing her potential to produce more litters annually (Cole et al., 1975). It has been demonstrated by Cole and associates (1975) that the peak annual sow productivity (piglets per sow per year) is reached when lactation length is between 20 and 28 days. At shorter lactation lengths the decrease in number of piglets per litter outweighs the advantage of an increased number of litters per year. Likewise, at longer lactations, the increase in number

of piglets per litter does not compensate for the prolonged farrowing interval and annual sow productivity declines (Cole et al., 1975). However, the practice of early weaning is very stressful for the piglet (Stanton and Mueller, 1976; Worsaae and Schmidt, 1980) and provisions must be made to ensure that stress is minimized and the animals remain healthy and productive.

The climatic physiology of the young pig has been well studied and it is of great concern at the time of weaning. Piglets have an underdeveloped thermoregulatory system and very little body fat at birth (Mount, 1968). Therefore, it is essential that supplementary heat is provided until thermal comfort can be maintained independently by the young pig. Newly weaned pigs are generally provided with supplementary heat delivered by infrared radiation, heated flooring (Barber et al., 1955), or hot air furnace. The cost of providing heat in these manners reduces the profit margin gained by early weaning (weaning at ≤3 weeks of age). More recently, another form of supplemental heat for livestock has been investigated. Microwave radiation has been shown to be effective in providing a suitable thermal environment for young pigs as determined by feed:gain ratio and by increased body weight (Braithwaite et al., 1994).

Microwave radiation has several advantages over traditional forms of supplemental heating systems. The cost of providing microwave radiation in terms of electricity is less than the cost of operating heating lamps or providing floor heating ($1.23 per day for 2 250 W infrared lamps compared with $0.32 per day for 128 W of microwave energy (Maritime Electric rates manual and policies schedule, 1999); both sufficient to maintain thermal comfort for at least 4 three-week-old pigs). Furthermore, the penetration capacity

of microwaves into the animal reduces the need to transfer heat internally as required with traditional forms of supplemental heat. Finally, the mode of action of microwave radiation is such that it generates heat within the animal by vibration of polar molecules (Copson, 1975). Therefore, thermal comfort is provided without wasting energy heating the air or the facility. The advantage of heating the animal directly is that the housing facility can be well ventilated, reducing ammonia and CO_2 concentrations, which results in improved animal and human wellbeing (Donham, 1991). Microwave radiation, as a form of supplemental heat, could mean tremendous savings for the swine industry.

The introduction of new technology such as microwave radiation raises concerns about safety for the target subjects as well as for the operator. Over the past 30 years much research has been conducted to determine the safety of microwave radiation and possible adverse side effects. As a result of these studies, guidelines on the recommended limits of exposure have been established by organizations such as the International Radiational Protection Association (IRPA, 1988) and the American National Standards Institute (ANSI, 1988). Many investigations have been carried out to determine thermal effects of microwave radiation as well as some athermal effects involving behavioural modifications (Korbel and Thompson, 1965; Korbel, 1969; Mitchell et al., 1977). Previous studies on the effects of microwave radiation on livestock have suggested a possible suppressive effect of 2450 MHz microwave radiation on activity level in weaner pigs (Braithwaite et al., 1992). However, the effect of microwave radiation at 915 MHz on activity level in weaner pigs has not been quantified.

The focus of these studies was to quantify activity level in newly weaned pigs

exposed to infrared radiation, as the standard heating system, and 2 levels of microwave radiation at 915 MHz. The frequency of 915 MHz was chosen for its greater penetrating capacity compared with 2450 MHz (3.04 cm vs 1.7 cm in muscle for 915 MHz and 2450 MHz, respectively) (Johnson and Guy, 1972). If a microwave effect was detected the second objective was to investigate the physiological basis of the behavioural modification.

1.2 Problem of early weaning

Pigs are raised mainly for meat production. Therefore, in any finishing operation, the onus is on getting as many pigs to slaughter weight as possible in the shortest possible time. In general, pigs are very efficient animals in that they are rapid growers and high producers. However, there is a constant demand for improving efficiency. Over the years, the demand for increased productivity has led to a reduction in the weaning age of piglets. While there is variability around the world with respect to age at weaning, in most countries weaning age varies from 2 to 8 weeks (English et al., 1988). Given that natural weaning of piglets is generally completed by approximately 14 - 16 weeks of age (Jensen, 1986), 2 - 8 weeks is very young for these animals. The act of weaning is a very stressful event for any animal. When this occurs at a time before the animal is prepared physically and physiologically, the added stress can be detrimental to the animal (Ader, 1962). Therefore, the early weaned piglet requires a great deal of extra care in terms of diet, housing, climate, health care, social requirements and overall management.

1.2.1 Dietary change

There are several factors to consider when determining the optimum age for weaning piglets. First of all, at weaning the piglet is suddenly deprived of its mother's milk and placed on a diet consisting of ingredients such as spray-dried porcine plasma, spray-dried blood meal, soybean meal, dried skim milk and dried whey (Nelssen et al.,1995). The effect of this dietary change will vary depending on the age of the pig at weaning. At two to three weeks of age the effect will be greatest because the piglet will have eaten very little creep food by this time. At eight weeks, there will be little effect because in the final week the piglet will be obtaining 70 - 80% of its food requirement from creep food (English et al., 1977). In addition, the ability to digest fat and protein develops with age in the young pig as digestive enzymes such as lipase and proteases develop (Cunningham and Brisson, 1955; Lloyd et al., 1957; Frobish et al., 1969). Lloyd et al. (1957) reported that the mean digestibility of long chain fatty acids by pigs is 37% at 3 weeks of age and 78% at 7 weeks of age. Therefore, as weaning age decreases so does the ability to digest the new starter ration, which can reduce weight gain in the newly weaned pig (Leibrandt et al. 1975). Furthermore, sow milk yield rises to a peak after three weeks of lactation and is maintained at that peak for two weeks, followed by a gradual decline after the piglets have reached five weeks of age (English et al., 1977). Therefore, with respect to dietary change, it appears as though weaning at five - six weeks of age is optimal for the piglet. Early weaning also means savings for the producer in sow food costs since the sow will require less food if she is not lactating. According to Whittemore (1993) the daily energy requirements of a lactating sow weighing 240 kg, nursing 10

piglets and producing 12 kg of milk daily is estimated at 106.3 MJ ME. This is considerably higher than the estimated requirement of a 200 kg pregnant sow at 34 MJ ME day^{-1} (Whittemore, 1993). Although some of these savings in food costs will be put back into the piglets with esimated daily requirements of 11.6 MJ ME for a 10 kg pig (Whittemore, 1993), the advantage is that the piglet will utilise the food more efficiently. In 1973, Burlacu et al. estimated that piglets (average weight 14.3 $\pm$ 2.1 kg) were able to synthesize protein with an average efficiency of 78.1% and fat with an average efficiency of 78.7% of the metabolizable energy for production.

1.2.2 Piglet immunity

Development of the immune system in the young pig is another important consideration at weaning. The pig is born with little protective immunity as there is virtually no *in utero* transfer of immunoglobulins from its mother (Porter, 1969; Porter and Hill, 1970). Therefore, it relies on the passive transfer of immunoglobulins (IgA, IgG, and IgM) from colostrum and mother's milk (Porter, 1969; Porter and Hill, 1970). It has been shown by Porter and Hill (1970) that colostrum derived IgM and IgA antibodies decline in the first week of life while IgG levels take 21 days to reach a minimum concentration. Furthermore, the piglet does not make significant amounts of its own antibodies until 3 weeks of age, except for IgM, which it begins to produce after the first week of life (Porter and Hill, 1970). Therefore, a pig that is weaned between 1 and 3 weeks of age is extremely vulnerable to infection due to the low level of immunity at this point in its life. This vulnerability is further compounded by the fact that the presence of

passively acquired immunoglobulins interferes with active production of immunoglobulins by the piglet (Porter and Hill, 1970). In addition to the lack of circulating immunoglobulins, the piglet is also losing the intestinal mucosal immunity provided by the IgA it receives from its mother's milk. Thus, not only is the piglet at risk of developing digestive problems due to a dietary change but it is also more susceptible to gastrointestinal diseases due to a lack of local immunity. Furthermore, it appears that the weaning of pigs prior to five weeks of age may reduce the cellular immune response (Blecha et al., 1983). On the other hand, vertical transmission of certain diseases such as enzootic pneumonia and atrophic rhinitis can be prevented through the implementation of programs such as segregated early weaning and medicated early weaning (Alexander et al., 1980). However, the success of these programs requires strict management to prevent the introduction of pathogens. Pigs in these systems would succumb to disease more easily than other pigs due to the lack of exposure and the opportunity to build up immunity against minor pathogens.

1.2.3 Effect of early weaning on subsequent litter size

Although the original motivation for early weaning of pigs was to increase productivity, there is a point where early weaning results in lower productivity. As weaning age decreases so does recovery time for the sow's uterus. The result is a uterus and an endocrine system that are less well prepared to support another pregnancy and therefore smaller subsequent litter size may result (Cole et al., 1975; Clark and Leman, 1987; Friendship, 1987; Dewey et al., 1994). In addition, the weaning to estrus interval may be longer when pigs are weaned before 3 weeks of age due to incomplete involution

of the uterus (Svajgr et al., 1974). It appears that from 21 days of lactation onward these effects are negligible and productivity would be maximised when weaning occurs between 21 and 28 days of lactation (Cole et al., 1975). Dewey and associates (1994) estimated that an increase of 0.16 pigs per litter would be expected for sows weaned at 20-24 days in lactation vs. weaning at less than 20 days. Also, an additional increase of 0.16 pigs per litter could be expected when sows are weaned at 25 or 26 days of age (Dewey et al., 1994).

1.2.4 Space and temperature requirements

Another consideration for the early weaned pig is space requirements. The earlier the piglets are weaned the less farrowing accomodations are required and the more weaner accomodations are needed in the production unit. Since weaners require less space than lactating sows ($0.16m^2$ - $0.21m^2$ per 10kg pig, depending on floor type, compared with recommended farrowing crate dimensions of 2.4m x 75cm) (Agriculture and Agri-Food Canada Publication, 1993), this may mean reduced housing costs for the producer. However, this saving may be counterbalanced by the additional requirements of piglets in terms of environmental temperature. Temperature requirements of newly weaned pigs vary depending on the weight of the animal at weaning. At 5-8kg (~3 weeks of age) piglets require an average environmental temperature of 28°C while between 8 and 10kg (~4 weeks of age) they require 25°C and from 10-15kg, they require 22°C (Mount, 1968). The cost of providing these high temperatures while maintaining good ventilation and adequate humidity (60-80% relative humidity)(Whittemore, 1993) can be quite high.

1.2.5 Social stress

The time of weaning for a piglet not only means being taken away from the protection and care of its mother but also being mixed with pigs from other litters resulting in the disruption of the social hierarchy established among littermates (McBride, 1963; Beilharz and Cox, 1967). The ensuing struggle to regain social order adds to the stress of weaning and may be detrimental to the health of the more subordinate individuals.

1.2.6 Effect of early weaning on growth

All of the stressors associated with weaning culminate in a very traumatic situation for the piglet resulting in an initial stasis in growth (Lucas et al., 1959; Stanton and Mueller, 1976). This growth stasis has been proposed by Whittemore et al. (1978) to be due to lipid loss rather than loss of protein. Total lipid content of the newly weaned piglet (2 - 4 weeks of age) was shown by Whittemore et al. (1978) to be approximately 15% at the time of weaning and was followed by a transient reduction to 7.6% and then recovery to 7.7-10.9% by 4 weeks postweaning. In the same study protein content of the pigs showed little change over the 4 week postweaning period. By 21 days of age the developing young piglet is beginning to undergo changes in digestive ability such as alterations in digestive enzyme production (Wilson and Leibholz, 1981; Hampson and Kidder, 1986) and changes in small intestinal crypt depth (Hampson, 1986). These developments enable the piglet to better survive on its own. Weaning prior to these developments may compromise the piglet's ability to adapt to the new diet and may cause the pig to succumb to disease. In fact, Hampson (1986) found that weaning at 3 weeks of

age resulted in greatly reduced villus height:crypt depth ratios. These changes in intestinal morphology resulted in a temporary reduction in the ability to absorb and digest nutrients. Therefore, the older the pig is at weaning, the better are its chances of survival.

1.3 Climatic physiology

Pigs are quite unique in comparison to other domestic species in terms of their climatic requirements. They have very little hair coat for protection against the elements and rely mainly on their body fat and behaviour to maintain thermal homeostasis (Mount, 1960; 1966; 1967; 1968). In addition, new born pigs appear to have no brown adipose tissue which is an important energy source in other domestic new born animals (Mount, 1968). In fact, the total fat content in new born pigs is ~ 1% of the body weight (Widdowson, 1950). As a result, the pig is quite susceptible to changes in environmental temperature beyond the upper and lower critical temperatures (UCT and LCT, respectively)(Mount, 1960). The UCT is defined as the temperature at which heat balance can be maintained only when all of the animal's thermoregulatory processes operate at maximum effectiveness (Curtis, 1983). The LCT is defined as the temperature at which insulative and behavioural responses to cold stress are at their maximal effectiveness in conserving heat (Curtis, 1983).

The thermoneutral zone for the pig is mainly determined by its body weight, thickness of the fat layer, energy intake and environmental conditions. The effect of body weight is such that higher ambient temperatures are required for thermoneutrality at lower body weights and vice versa (Heitman and Hughes, 1949; McCracken and Caldwell,

1980). In addition, pigs of similar body weight but different thickness of backfat have different effective environmental temperature requirements as demonstrated by Henken et al. (1991). In that study, backfat thickness was negatively correlated with LCT in growing pigs. However, temperature requirements are also influenced by the amount of heat produced by the pig through energy metabolism and therefore, by the amount of food intake (Verstegen et al., 1973). Verstegen and associates (1973) demonstrated that at 20°C pigs lose more heat when consuming 45 g food kg^{-1} BW per day than those consuming 39 g food kg^{-1} BW per day. Naturally, as more food is consumed, heat production increases and heat must be dissipated to the environment. In such situations, a lower ambient temperature would be desirable. On the other hand, if food intake is low, metabolic energy for heat production is decreased. Without a compensatory increase in ambient temperature, the pig will utilize more of its energy intake for heat production at the expense of growth. Verhagen et al. (1987) demonstrated that the maintenance requirements for 10 week old pigs kept at 15° C was higher than that for pigs kept at 25°C. Furthermore, the energy available for gain was lower in the pigs kept at 15°C than those kept at 25°C. The temperature range for thermoneutrality in a group of weaner pigs (~5 kg liveweight) consuming only enough food for maintenance has been estimated by Mount (1968) to be between 27°C and 34°C. Within this temperature range, piglets use behavioral thermoregulatory mechanisms to maintain homeostasis. However, at temperatures above and below this range additional thermoregulatory mechanisms are activated (Mount, 1968).

1.3.1 Effect of environmental conditions on temperature requirements of pigs.

Environmental conditions such as air movement, floor type, stocking density and wet lying areas also influence the thermal environment of the pig. For example, increasing the air speed in a pig unit to 0.5 m sec^{-1} is equivalent to decreasing the effective environmental temperature (EET) by 3°C and a further increase in air speed to 1 m sec^{-1} will reduce the EET another 3°C (English et al., 1988). Flooring type is very important because if insulated flooring is utilized pigs can be reared comfortably at low average ambient temperatures (3.48°C)(McLagan and Thompson, 1950) and this effect is further increased if dry bedding is provided (Mount, 1967; Stephens, 1971). If the bedding or flooring is wet, however, the LCT will be increased drastically. Similarly, when slatted flooring is used, the draught from underneath can increase the LCT but the advantage is that there is no buildup of moisture so the animals stay dry. Finally, the stocking density can influence the ambient temperature of a pig unit through the production of heat energy by a greater number of pigs (Mount, 1960).

1.3.2 Thermoregulatory mechanisms

Pigs have a deep body temperature of 39°C which they will attempt to maintain at all costs. If they are reared under conditions in which temperature remains within the comfort zone, they do not have to expend energy to stay warm or dissipate heat to stay cool. Behavioural thermoregulatory mechanisms are used to maintain comfort within this temperature range. However, if environmental temperature drops below that temperature at which maximal metabolic rate is achieved (i.e. below the LCT), deep body temperature

will drop and death may ensue due to hypothermia (Mount, 1968). On the other hand, if the ambient temperature rises above the temperature at which metabolic rate is at a minimum (i.e. above the UCT), the pig will attempt to dissipate heat through mechanisms such as panting which will increase its metabolic rate. This may result in increased heat production and spiral hyperthermia ensues (Mount, 1968). When faced with environmental temperatures beyond the level of thermoneutrality, the pig will utilize physiological and behavioural mechanisms to maintain a constant deep body temperature. At low temperatures an increased metabolic rate (Stephens, 1971; Henken et al., 1991), peripheral vasoconstriction, shivering (Stephens, 1971), pilo-erection, and postural changes (Mount, 1967; Stephens, 1971) will effectively increase heat production and/or decrease heat loss. At high temperatures a decreased metabolic rate (Henken et al., 1991), sweating, wallowing, and postural changes (Stephens, 1971) will result in decreased heat production and increased heat loss to the environment.

1.3.3 Methods of heat loss from the pig

There are four methods by which the pig loses heat to its environment: radiation, conduction through contact with surrounding objects, convection from the body surface, and evaporation of water from the lungs or from the body surface such as water, mud or urine. At temperatures below the LCT evaporative losses are at a minimum and additional heat loss occurs by conduction, convection and radiation (English et al., 1988). In an attempt to conserve heat, the pig will assume a posture such that there is minimal body contact with the floor (i.e. it will lay in sternal recumbency with its feet tucked underneath

its body) (Mount, 1967). This will reduce the conductive heat losses. Also, groups of pigs will huddle together to reduce heat loss to the surroundings (Mount, 1960). At temperatures above the LCT, the pig will increase its evaporative heat loss, decrease its non-evaporative loss and peripheral vasodilation will result in reduced resistance to the outward flow of heat. A more relaxed posture will be adopted and body contact with the floor will be increased (Stephens, 1971). Pigs kept in groups will space themselves out while still maintaining contact with one another. As the UCT is approached and passed, the pig, if given a chance, will wallow in water (Garrett et al., 1960; Heitman et al., 1962), mud, feces or urine (Heitman and Hughes, 1949). This will increase heat loss from the body surface through conduction or evaporation. Body contact with cool surfaces will be maximized and groups of pigs will be well spaced out avoiding contact with one another. Under extreme conditions, the pig will also pant to increase evaporative losses (Christon, 1988).

1.3.4 Effect of inadequate environmental temperature on growth.

The pig's attempt to maintain euthermia under adverse environmental temperatures will have a negative effect on its growth and feed conversion efficiency (Verhagen et al., 1987; Lopez et al., 1991a;b; Becker et al., 1992). In order to gain weight the pig must consume more food energy than it requires for maintenance. The more energy that is used to maintain body temperature, the less will be available for growth. As the energy requirements for heat production exceed the energy consumption, the pig will begin to catabolize body fat and protein for maintenance and will start to lose weight. Therefore,

for the same amount of food intake the pig may gain weight at the thermocomfort level and lose weight or grow more slowly at temperatures at or below the LCT (Verhagen et al., 1987). In the study by Verhagen et al (1987), pigs kept at 15°C showed a decrease of 60g in daily gain over a 6 day period compared with pigs of similar weight kept at 25°C. Furthermore, maintenance requirements of pigs kept at 15°C was signifiantly increased compared with that of pigs kept at 25°C. On the other hand, at temperatures above the UCT, the pig will have to dissipate heat to avoid heat stress. If heat loss is insufficient to maintain constant deep body temperature, the pig will reduce its food intake in order to reduce its metabolic heat production (Christon, 1988; Lopez et al., 1991a; Becker et al., 1992). This will result in less energy available for weight gain. In 1991, Lopez and colleagues demonstrated a reduced average daily gain (ADG) of 17.6 g day^{-1} by pigs kept in a hot diurnal temperature of 22.5-35°C compared with pigs kept at a constant temperature of 20°C.

A good understanding of the climatic requirements of the pig is very important in order to maximize productivity from a herd. The optimum weight gain and food conversion efficiency will be achieved at minimal cost when metabolic heat production is at a minimum and the need for heat dissipation is eliminated through the provision of a suitable environment.

1.4 Behaviour patterns of domestic pigs

The behaviour of domestic pigs has been studied at great length over the past 40 years (McBride et al., 1964; Hafez and Signoret, 1969; Ewebank and Meese, 1971)

providing valuable information that can be used to identify problems in pig rearing systems. By understanding the normal behaviour of an animal under certain conditions, one can identify and use abnormal behaviours to assess the animal's current environment. Changes can then be made to the environment so that the animal is more comfortable and can engage in normal behaviours. Some behaviours that are commonly used as indicators of discomfort or stress in pigs are social behaviour (Ewebank and Bryant, 1972), activity level (Meunier-Salaün et al., 1991), and eating and drinking behaviours (Rushen, 1984; Terlouw et al., 1991).

1.4.1 Social behaviour

Pigs are very social animals and tend to live in herds of less than 10 individuals in the wild (Kurz and Marchinton, 1972). As is the case with most group-oriented species, pigs have a well-defined social hierarchy that provides structure to the group and allows the harmonious interaction of its members (Meese and Ewebank, 1973). Under present production conditions, the dominance hierarchy is established after weaning when pigs from different litters are mixed for the first time. During the first 24 hours, most of the interactions are agonistic and the animal that wins the most fights emerges as the top-ranking animal (Meese and Ewebank, 1973; Friend et al., 1983). This dominant pig will have precedence over all the other pigs in the group when competing for resources such as food or mates. It has been found that the hierarchy formed by pigs is generally a linear one, with A being dominant over B which is dominant over C, etc. (Rasmussen et al., 1962). However, occassionally two pigs may occupy the same social rank or special

relationships such as triangular hierarchies, may be formed in which A > B > C > D > E > F > G but F dominates D (Ewebank, 1969).

Agonistic interactions between pigs follow very specific patterns. Fighting behaviour between sows and young pigs generally consists of biting directed at the head, neck and shoulders (Fraser, 1974). The ears are especially favored targets. Young pigs will sometimes ram their opponents with their heads (Fraser, 1974). Eventually, one of the opponents will submit to the other as indicated by the tilting of the head away from its adversary (Jensen, 1982), a single step back or a rapid retreat which may be accompanied by vocalization (Signoret et al., 1975). Once the dominance-subordinance relationship has been established between pigs, communication of threat or submission usually consists of a simple glance in an opponent's direction or the lowering of the eyes in submission (McBride et al., 1964).

1.4.2 Activity patterns

The activity pattern of adult feral swine is typically diurnal in the winter months and nocturnal in the summer months (Kurz and Marchinton, 1972), consisting of activities such as exploring, rooting (if conditions permit), eating, drinking, nibbling and chewing. In confinement rearing of pigs, occurrence of these activities is minimal and, if the environment is satisfactory, the pig will sleep for up to 80% of the day (Van Putten, 1969). However, if the animal is uncomfortable it will become restless and engage in abnormal behaviour such as tail biting (Van Putten, 1969).

1.4.3 Feeding and drinking behaviour

Pigs are omnivorous animals eating anything from plants, seeds, roots and grass to worms, slugs, snakes, rodents and even ill or dead animals (Signoret et al., 1975). If kept outside, they will spend up to 6-7 hours per day foraging for food (Signoret et al., 1975). When reared inside and hand fed concentrates, pigs can spend less than 10 mins per day eating. However, when food is readily available *ad libitum*, feeding time is increased (Signoret et al., 1975). Pigs will often eat as a group even when self feeders are used because the movement of one pig to the feed area will stimulate others to eat as well through social facilitation. Eating is usually alternated with drinking unless the animals are hand fed. In the latter case all the food is finished before consuming any water.

1.5 Characteristics of mammalian sleep

1.5.1 Definition of sleep

The subject of sleep has been studied for many years and although much has been learned about the physiological state of the mind and body during sleep, it still remains something of a mystery. Sleep is obviously an essential part of life since it has been carried through evolution and occupies a large portion of mammalian life in spite of the vulnerability associated with the sleep state. In fact, sleep disorders can severely disrupt a person's life (Portaluppi et al., 1994) and the complete absence of sleep in rats has been shown to result in death (Rechtschaffen et al., 1983; Gilliland et al., 1989). According to Collin's English Dictionary (1991), sleep is defined as "a periodic state of physiological

rest during which consciousness is suspended and metabolic rate is decreased". This definition implies that the purpose of sleep is to provide a rest period for the body and mind, a theory that has been advocated by several investigators of sleep (Hess, 1954; Moruzzi, 1972; Oswald, 1980). Other theories put forth to explain the function of sleep include facilitation of the immune response (Krueger et al., 1989), and avoidance of sustained heat loads (McGinty and Szymusiak, 1990).

1.5.2 Sleep structure

The structure of sleep has been described based on electroencephalographic (EEG) measurement of brain electrical activity and human sleep generally consists of five stages (Webb, 1971): four stages of slow wave sleep (SWS) and one stage of rapid eye movement (REM) sleep. The four stages of SWS are characterized by progressively slower frequencies and higher voltage activities and correspond to successively deeper states of sleep (Kelly, 1991). Rapid eye movement sleep is characterized by the following features: 1) desynchronized electrocortical activity, 2) theta rhythm within the hippocampus, 3) pronounced atonia of the postural muscles, 4) field potentials in the pons, lateral geniculate nucleus, and occipital cortex (ponto-geniculo-occipital or PGO spikes), 5) rapid eye movements, and 6) myoclonic twitches, most apparent in the facial and distal limb musculature (Vertes, 1990). Although primates have a very similar sleep structure to that of humans, characterized by five stages with essentially identical EEG criteria as those of humans (Freemon, 1972), most other species differ in sleep structure. Rats have two stages of sleep: SWS and paradoxical sleep (PS) (Van Twyver, 1969). Paradoxical sleep is a term

which refers to the stage of sleep characterized by desynchronized cortical EEG, muscle atonia, and hippocampal theta waves (Webb and Dube, 1981). Since some animals do not exhibit rapid eye movements, this "activated" sleep stage is referred to as paradoxical sleep rather than REM sleep (Webb and Dube, 1981). Birds, like rats, have two stages of sleep: SWS and PS but the episodes of SWS are shorter than in mammals and PS does not exceed 10% of total sleep time (Ookawa, 1972). Cats, on the other hand, have two stages of SWS and one stage of PS (Ursin, 1970) and ruminants spend a significant amount of time in a state of "drowsiness" (Ruckebusch, 1972). Pigs also spend a notable portion of their day (16%) in a state of drowsiness which is characterized by a complete loss of muscle tone and has been reported to resemble the state of SWS, superficially (Robert and Dallaire, 1986). Robert and Dallaire (1986) also reported that pigs spend ~27% of a 24 h period in SWS and ~11% of a 24 h period in PS. Ruckebusch (1972) reported that pigs spend ~21% of a 24 h period in a state of drowsiness, ~25% of a 24 h period in SWS and 7.3% in PS. Only 46% of the pig's 24 h period was spent in alert wakefulness (Ruckebush, 1972).

1.5.3.Sleep physiology

1.5.3.1 Sleep Centers

The desire to understand how and why we sleep has led to the development of various experimental protocols ranging from the noninvasive measurement of brain electrical activity (Dement, 1958; Rechtshaffen et al., 1983), to the very invasive brain transection experiments in animals (Bremer, 1935; Jouvet, 1972). Through a series of

transection experiments (Bremer, 1935, 1937, 1938; Jouvet, 1972; Moruzzi, 1972; Zernicki, 1988), the brainstem has been identified as the major control center for sleep. Bremer (1935; 1937; 1938) demonstrated that transection of the brainstem from the spinal cord (encephalé isolé preparation) did not alter the normal sleep-waking cycle while transection of the forebrain from the midbrain (cerveau isolé preparation) resulted in a permanent state of sleep. Bremer attributed the permanent sleep state of the cerveau isolé preparation to lack of sensory stimulation of the forebrain. By transecting the brainstem cranial sensory nerves in an encephalé isolé preparation Bremer further demonstrated that the sensory input of the cranial nerves caudal to this midbrain transection were necessary for the maintenance of wakefulness. The result was similar to the cerveau isolé preparation; a constant state of somnolence (Bremer, 1937). The importance of sensory input to the forebrain in the maintenance of wakefulness was later confirmed by Moruzzi and Magoun (1949) with the demonstration of a tonically active ascending reticular activating system (ARAS) within the upper pons and midbrain. Sensory input into the ARAS activates the cerebral cortex resulting in electroencephalographic activity that is consistent with a state of wakefulness. Subsequently, brainstem transections performed just caudal to the ARAS (midpontine pretrigeminal preparation) (Batini et al., 1958, 1959a, 1959b; Zernicki, 1986) resulted in a constant state of wakefulness which led to the theory that sleep occurred as a result of active inhibitory input to the ARAS from the caudal brainstem and this inhibition was eliminated in the pretrigeminal preparation. In 1961, two separate studies (Favale et al., 1961; Magnes et al., 1961) confirmed that low frequency (1-16 Hz) electrical stimulation of neurons in the medullary region of the brainstem would

induce electrocortical synchronization or sleep. Subsequent experiments revealed that areas of the basal forebrain are also involved in the induction of sleep. Sterman and Clemente (1962a) demonstrated that low frequency stimulation of a limited region of the basal forebrain, the anterior hypothalamus/medial preoptic area, resulted in widespread electrocortical synchronization. In a follow-up study, Sterman and Clemente (1962b) showed that bilateral stimulation of the same area resulted in cortical synchronization and sleep in freely moving cats. Furthermore, a group of neurons has been identified in the basal forebrain area which discharge at a faster rate during EEG synchronization and sleep than during EEG desynchronization and wakefulness (Mallick et al., 1983; Kaitin, 1984; Szymusiak and McGinty, 1986, Ogawa and Kawamura, 1988). These studies suggest that there are two areas in the brain which are responsible for the sleep phase of the mammalian sleep-wake cycle; one in the basal forebrain and one in the medullary region of the brainstem. In fact, Mohan Kumar and associates (1985) demonstrated that stimulation of the lower medullary neurons responsible for sleep resulted in excitation of the basal forebrain neurons that are involved in sleep induction, suggesting that the two sleep centers work together to induce sleep.

1.5.3.2 Sleep inducing agents

Having identified the areas of the brain that are responsible for sleep induction, the next problem that needed to be addressed was identification of the substance (neurotransmitter or hormone) that was responsible for the activation of the sleep-active neurons (ie. those neurons that fire more rapidly during sleep than during wakefulness).

Early studies by Jouvet and colleagues (1966) suggested that 5-hydroxytryptamine (5-HT or serotonin) may be involved in the induction of sleep. Petitjean et al. (1985) showed that the insomnia induced by administration of parachlorophenylalanine (PCPA) (an inhibitor of tryptophan hydroxylase, an enzyme necessary for the synthesis of 5-HT) in cats could be reversed by systemic, intraventricular or intracisternal injection of 5-hydroxytryptophan (5-HTP) (a precursor of serotonin). Subsequently, it was shown that the hypnogenic effects of 5-HTP could be induced when it was injected into the rostral and ventral hypothalamus, in the medial preoptic area (Denoyer et al., 1989). Furthermore, destruction of raphe neurons (neurons found in the raphe nucleus, located in the brainstem), which are serotonergic neurons, by Jouvet et al. (1966) and Jouvet and Renault (1966) resulted in a complete loss of SWS for 3-4 days followed by minimal recovery up to 13 days postlesion. However, contradictory to the role of serotonin in sleep induction is the finding that unit activity of 5-HT perikarya (McGinty et al., 1973) and the release of 5-hydroxyindoleacetic acid (5-HIAA) from 5-HT terminals (Cespuglio et al., 1981) is decreased during sleep and increased during wakefulness. This finding suggests that serotonin is involved in the maintenance of wakefulness rather than the induction of sleep. Jouvet (1988) disputes this argument with the hypothesis that the release of serotonin during wakefulness controls events that initiate SWS. In addition, Dement et al. (1972) demonstrated that, although chronic administration of PCPA in cats resulted in an initial insomnia, this insomnia lasted for only 2-3 days and was followed by a return of approximately normal levels of sleep while 5-HT levels remained reduced by 90-95%. Several other sleep-promoting factors have been identified including muramyl peptides (Garcia-Arraras and Pappenheimer,

1983), lipopolysaccharides, prostaglandins (Hayaishi, 1988), interleukin-1, interferon-α2, tumor necrosis factor, delta sleep-inducing peptide, and vasoactive intestinal peptide. These factors also have an effect on body temperature and the immune response which has led to the theory that another important function of sleep is to optimize the processes that help to eliminate infections.

1.5.4 Control of the sleep-wake cycle

Various mammalian activities and endocrine functions occur in rhythmic fashion which are repeated over a 24-hour period (i.e. they exhibit circadian rhythms)(Halberg, 1969). The sleep-wake cycle is among those rhythms that occur in a daily pattern of cyclicity. In 1967, Richter identified a neuroanatomic site in the mammalian brain which is responsible for controlling circadian rhythms such as activity, feeding and drinking. This site was later confirmed to be the suprachiasmatic nucleus (SCN) which is a paired structure of the hypothalamus located directly above the optic chiasm. The importance of the SCN in the maintenance of circadian rhythms was demonstrated by the loss of several circadian rhythms such as adrenal corticosterone secretion in rats (Moore and Eichler, 1972) and drinking and locomotor activity in hamsters (Stephan and Zucker, 1972) following experimental destruction of the SCN. The sleep-wake cycle is also under the control of this hypothalamic structure as demonstrated by the disruption of sleep-wake pattern by lesions in the SCN (Ibuka and Kawamura, 1975; Coindet et al., 1975; Ibuka et al., 1977; Stephan and Nuñez, 1977). In addition to the disruption of the sleep-wake cycle, lesions of the SCN also disrupt circadian rhythms of body temperature (Stephan and

Nuñez, 1977), locomotor activity (Stephan and Nuñez, 1977), and heart rate rhythm (Saleh and Winget, 1977).

Rhythmic neural activity within the SCN appears to be an inherent property of the nucleus itself since this rhythm persists upon isolation of the nucleus from the surrounding tissue by knife cuts (Inuoye and Kawamura, 1979). However, it appears that certain external cues such as the light-dark cycle can entrain endogenous rhythms (Czeisler et al.,1981; 1986). Such external cues are termed zeitgebers which, translated from German, means 'time givers'. These zeitgebers require a route by which they can reach the SCN and set the circadian rhythm. In 1972, this route was elucidated by the work of Moore and Lenn who demonstrated the existence of a monosynaptic retinohypothalamic tract which terminates in the SCN of the hypothalamus. This tract was identified by injecting tritiated amino acids into the vitreous humor of the eye.

1.5.5 Regulation of melatonin synthesis and metabolism

Melatonin (5-methoxy-N-acetyltryptamine) is an indole derivative produced primarily in the pineal gland and secondarily in the retina, harderian gland (Pang et al., 1977), the small intestine (Raikhlin et al., 1975) and blood platelets (Launay et al., 1982). The pineal hormone was discovered in 1958 by Lerner and collegues while searching for the amphibian skin lightening pigment found in bovine pineals.

The synthesis of melatonin begins with the uptake of the amino acid, tryptophan, from circulation and its conversion to 5-hydroxytryptophan by *tryptophan-5-hydroxylase* within the pineal gland. The next step is the decarboxylation of 5-hydroxytryptophan to

serotonin by the enzyme *aromatic amino acid decarboxylase*. Serotonin is then converted to N-acetylserotonin in the rate limiting step which requires the enzyme, *N-acetyltransferase* (NAT) (Weissbach et al., 1961). Finally, melatonin is produced upon O-methylation of N-acetylserotonin by the enzyme, *hydroxyindole-O-methyltransferase* (HIOMT) (Axelrod and Weissbach, 1960). Melatonin synthesis within the pineal gland is controlled by neural signals from the suprachiasmatic nucleus which regulate the enzymes, NAT and HIOMT (Klein and Moore, 1979). Norepinephrine release from the superior cervical ganglion is responsible for the activation of NAT and HIOMT (Klein and Moore, 1979). Klein and Moore (1979) have also demonstrated that light stimulus is the external cue responsible for regulating these pineal enzymes via the retinohypothalamic tract. By transecting all components of the primary and accessory optic tracts and leaving only the retinohypothalamic tract intact, Klein and Moore (1979) demonstrated that NAT and HIOMT were suppressed by light stimulus. Furthermore, they showed that exposure to light during the dark period resulted in a rapid decrease in NAT activity which did not occur in animals in which the retinohypothalamic tract had been transected. Binkley et al. (1974) had previously shown that pineal NAT activity increased in the dark resulting in increased synthesis of melatonin during the dark phase of the light-dark cycle.

Following the synthesis of melatonin in the pineal gland, the hormone is released into the circulation and also into the cerebrospinal fluid (Brown et al., 1979). Metabolism of melatonin occurs primarily in the liver where it undergoes 6-hydroxylation followed by sulfate or glucuronide conjugation and is subsequently secreted in the urine (Kopin et al., 1961). It has been demonstrated by Kveder and McIsaac (1961) that exogenously

administered melatonin is eliminated from the circulation by a 2-step process with half-lives of approximately 2 and 45 minutes each in mice. Subsequent studies have demonstrated that exogneously administered melatonin in humans is also eliminated by a 2-step process with plasma half-lives of approximately 3 and 45 minutes each (Bojkowski, 1988).

1.5.6 Relationship between melatonin, sleep and thermoregulation

Studies investigating the control of the circadian rhythmicity of sleep have led to the discovery that the pineal hormone, melatonin, plays an important role in the sleep-wake cycle. Melatonin was first shown to have a sleep-inducing effect in 1964 when Marczynski and collegues implanted melatonin in the hypothalamus of unrestrained cats. Within 15-30 min.of direct application of melatonin to both preoptic anterior hypothalamic regions, cats showed synchronized cortical EEG and sleep. Subsequently, Barchas et al.(1967) observed a sleep-like state in mice following intravenous melatonin administration. In 1969, Hishikawa and collegues provided further support for the sleep-inducing effect of melatonin with the demonstration of behavioural and electrographic patterns of slow-wave sleep in young chicks following intraperitoneal injection of melatonin. Numerous investigators have since reported similar findings of the somnolence inducing effect of melatonin in humans (Anton-Tay et al., 1971; Cramer et al., 1974, Vollrath et al., 1980). Furthermore, in 1994, Dollins et al., reported a decreased latency to sleep onset as well as an increase in sleep duration following melatonin administration in healthy male volunteers. The circadian rhythm of melatonin secretion that has been

reported in many species (Lynch, 1971; Ozaki et al., 1976; Rollag and Niswender, 1976; Hedlund et al., 1977; Tamarkin et al., 1979; Illnerova et al., 1985) is coupled to the sleep-wake cycle in that peak melatonin concentrations are obtained during sleep and low levels are present in the circulation during waking hours. In fact, the onset of sleep in young and elderly men has been shown by L'Hermite-Baleriaux et al. (1989) to be preceded by a rise in blood melatonin levels by one hour. Portaluppi and collegues (1994) provided further evidence of the relationship between the circadian rhythm of plasma melatonin and sleep by demonstrating that, in patients with fatal familial insomnia, as the disease progressed (i.e. sleep time declined) the circadian rhythm of plasma melatonin became disrupted until complete eradication of the rhythm was achieved.

Body core temperature has been associated with sleep in several studies (Aschoff and Wever, 1981; Barret et al., 1987; Wever, 1989). However, it has also been shown that the nocturnal decrease in body temperature is dissociable from sleep (Aschoff and Wever, 1981; Moore-Ede et al., 1983; Czeisler et al., 1986; 1989; Barret et al., 1987; Wever, 1989) and that it will persist at a lower amplitude in sleep-deprived subjects (Aschoff and Wever, 1981; Barret et al., 1987). Furthermore, the nocturnal decline in body core temperature is inversely correlated with the rise in serum melatonin levels (Cagnacci et al., 1992). This suggests that perhaps the nocturnal rise in melatonin secretion may be influencing the circadian rhythm of body core temperature. In fact, human subjects working a nightshift schedule for 2 months demonstrated shifts in the diurnal rhythms of melatonin secretion and body core temperature such that the two rhythms remained coupled. In these subjects the rise in serum melatonin remained coupled with the decline

in body core temperature although these rhythms had shifted to coincide with the daytime sleep period (Cagnacci et al., 1992). In an effort to establish a causal relationship between melatonin secretion and body core temperature, Cagnacci et al. (1992) administered melatonin to female human subjects. The result was a reduced body temperature thus implicating melatonin as a physiological regulator of body core temperature. In fact, the anterior hypothalamus is known to be the main center for the regulation of body temperature (Boulant, 1981) and melatonin receptors have been identified in the human hypothalamus (Reppert et al., 1988; Morgan and Williams, 1989).

As with melatonin and sleep, however, melatonin and body temperature can be dissociated as shown by the administration of atenolol (a melatonin inhibitor due to its β-blocking activity) which resulted in constant nocturnal body temperature until the onset of sleep (Cagnacci et al., 1992). The rapid decline in body core temperature with sleep indicates that sleep may be responsible for temperature regulation independent of melatonin. These studies considered together seem to suggest that sleep, melatonin and body core temperature work as an integrated system in which one component will compensate for the absence of another to maintain homeostasis. Indeed, Romijin (1978) proposed that the pineal gland "acts as a general tranquillizing organ on behalf of homeostatic equilibrium in close relationship with changing environmental conditions". Furthermore, Cagnacci et al., in 1993 found that the nocturnal decline in body core temperature in women could be reduced by light exposure suggesting a melatonin mediated effect of light on body core temperature. This was further supported by the findings of Myers and Badia (1993) who demonstrated that exposure of male humans to varying 'high'

light intensities resulted in higher body temperatures than exposure to 'low' light intensity. However, their results did not indicate a dose-response relationship. Shanahan and Czeisler (1991) also demonstrated that the timing of the plasma melatonin rhythm can be shifted by a light stimulus and this shift will be accompanied by a corresponding shift in the temperature cycle.

Thus far, studies have been limited to the relationship between increased melatonin and corresponding decreases in body temperature. The literature provides no information relating increased body temperature and melatonin production. It is possible that this hormone is the mediator that restores body core temperature to homeostatic levels. Furthermore, the strong relationship between sleep and melatonin secretion suggests that sleep is an important component in thermoregulation. In fact, there have been many studies linking increased body or brain temperature and the duration of sleep (Sakaguchi et al., 1979; Szymusiak et al., 1991; Morairty et al., 1993).

1.5.7 Melatonin secretion in pigs

As stated previously, mammalian plasma melatonin follows a circadian rhythm of high levels during dark hours and low levels during light hours. This is true for most mammals (both nocturnal and diurnal species) except for the domestic pig (Reiter, 1987). Studies by McConnell and Ellendorff (1987), Reiter et al. (1987) and Minton et al. (1989) revealed that pigs do not exhibit a nocturnal increase in plasma melatonin during long photoperiods (i.e. spring and summer). Similar results were found by Brandt et al. (1986) for short day seasons (i.e. autumn and winter). However, McConnell and Ellendorff

(1987) and Minton and Cash (1990) reported a nocturnal rise in melatonin among approximately one half of experimental pigs when exposed to an equatorial (12 hour light) photoperiod. In an attempt to alter plasma melatonin concentrations in pigs Griffith and Minton (1992) exposed barrows to high light intensity (approximately 1800 lx) and to low light intensity (approximately 113 lx). They found that 1800 lx was sufficient to raise nocturnal melatonin levels compared to daytime levels but 113 lx had no effect on nocturnal plasma melatonin concentrations.

Although pigs do not exhibit a circadian rhythm of melatonin concentration, this hormone appears to play a significant role in the onset of puberty in these animals. In a study by Diekman et al. (1991) oral administration of 3 mg of melatonin (fed in diet) at 1530h induced early onset of puberty in prepubertal gilts. This effect was seen during both increasing day length and decreasing day length. However, the length of the estrous cycle was not affected although the animals continued to ingest melatonin during estrous. In addition, melatonin administration did not affect the average daily gain of pigs.

1.5.8 Melatonin and electromagnetic waves

Another interesting factor that has been found to have an effect on both plasma and pineal melatonin is extremely low frequency (ELF) radiation. Kato et al. (1993) suppressed plasma and pineal melatonin concentrations in albino (Wistar-King) rats by exposing them to circularly polarized (rotating vector) 50-Hz magnetic fields. In a subsequent study it was established that the effect was seen at 1 μT and above but not at 0.2 μT (Kato et al., 1994a). However, exposure to horizontal or vertical 50-Hz , 1 μT

magnetic fields did not alter melatonin levels in the blood or the pineal gland of albino rats (Kato et al., 1994b). Kato et al. suggested that the radiation may be acting directly on the pineal gland to produce electric (eddy) currents which suppress production of melatonin. They also suggested that the magnetic field may be having an indirect effect via the retinal photoreceptors. In this way, the induced electric field would be performing a function similar to that which occurs when light strikes the retina, producing a conformational change in the photoreceptor pigment rhodopsin. Microwave radiation is also a form of electromagnetic radiation which could possibly influence the circadian rhythm of melatonin secretion. However there is currently no known scientific exploration into this area of electromagnetic energy with respect to melatonin.

1.6 Microwave radiation

1.6.1 Generalities

Microwave radiation is a form of nonionizing electromagnetic (EM) radiation which ranges in frequency from 300 MHz to 300 GHz (Scott, 1993). It consists of 3 frequency bands known as the ultra high frequency (UHF) band, the super high frequency (SHF) band and the extremely high frequency (EHF) band in increasing order of frequency (Scott, 1993). Microwaves are produced by the acceleration of electric charges and consist of oscillating electric and magnetic fields which are oriented at right angles to one another and to the direction of propagation (Serway, 1986). The resulting waves may vary in

frequency, period, wavelength, amplitude, impedance, power or phase. These properties provide the basis by which EM waves are characterized.

1.6.2 Characteristics of electromagnetic waves

1.6.2.1 Frequency

The number of EM waves that pass a given point in one second is referred to as the frequency of the radiation and is expressed in cycles per second, or Hertz (Hz). The frequency most commonly used in heat applications is 2450 MHz (2450×10^6 Hz) (Scott, 1993). However, recently the frequency of 915 MHz has been investigated as a potential source of heat for animals (D'Andrea et al., 1980; Bate et al., 1992).

1.6.2.2 Period

The period of an EM wave is the time between the passing of 2 waves at a given point in space. It is equivalent to the reciprocal of the frequency (Scott, 1993).

1.6.2.3 Wavelength

The wavelength of an EM wave refers to the distance which corresponds to one period of the wave (Scott, 1993). The free-space wavelengths of EM waves produced at 915 MHz and 2450 MHz are 32.8 cm and 12.2 cm, respectively (Johnson and Guy, 1972).

An EM wave travelling through free space at any frequency has a velocity of 3 x 10^8 m s^{-1} (the speed of light) (Scott, 1993). Velocity, frequency and wavelength are related by the equation:

$$\text{Velocity } (v) = \text{frequency } (f) \times \text{wavelength } (\lambda)$$

Generally, the frequency at which EM waves are produced is the frequency at which they remain. Therefore, if the velocity of a wave is changed, as it occurs upon encountering a dielectric material, the wavelength must change proportionately in order to maintain the original frequency (Serway, 1986).

1.6.2.4 Impedence

The ratio of the electric field to the magnetic field expressed in units of ohms describes the impedence of any given wave. A wave travelling through free space has an impedance of 377 ohms (Scott, 1993).

1.6.2.5 Power

The power of an EM wave refers to the strength or amplitude of the wave and is the product of the electric and magnetic fields expressed in units of watts (W) (Scott, 1993). The amplitude of the wave refers to the maximum displacement of a wave travelling in a given direction. When the power of a wave is distributed over a specific

area it is expressed as incident power per unit area, or power density, in W m^{-2} or mW cm^{-2} (Michaelson and Lin, 1987).

1.6.2.6 Phase

Microwaves can also be characterized by the phase of one wave relative to another. The phase is used to describe the time difference between 2 electrical signals in units of degrees, with 360° equivalent to a time difference of 1 period (Scott, 1993).

1.6.3 Microwave propagation

Microwaves generally cannot be conducted through wire in the way that electricity is conducted (Scott, 1993). Instead, microwaves travel through space in wavelike fashion. In order to transmit microwaves without losing the energy through scatter radiation, a confined system must be employed. This type of wave propagation in which the waves are completely confined is known as guided propagation. Microwaves can also be transmitted through space from a transmitting antenna to a receiving antenna, called unguided propagation (Michaelson and Lin, 1987).

Waveguides and coaxial cables are two devices used to guide waves. One of the most common forms of waveguide is a hollow metal pipe, usually rectangular, which carries EM waves in the same way that a water pipe carries water (Scott, 1993). Within a waveguide only two types of wave can be propagated, those of the transverse electric (TE) mode and those of the transverse magnetic (TM) mode (Michaelson and Lin, 1987). A mode is a unique distribution of the fields of an EM wave. The TE mode has the electric

field oriented in the transverse plane only, while the magnetic field can exist in either the transverse or axial plane. Conversely, in the TM mode, the magnetic field may only exist in the transverse plane while the electric field can be oriented either transversely or axially. For any waveguide there is a particular frequency below which no modes will be propagated. This frequency is called the mode cutoff frequency and is determined by the dimensions of the waveguide (Scott, 1993).

A coaxial cable consists of an inner conductor, an outer conductor, and an insulator filling the space between the conductors. Microwaves are carried through the insulator of the cable and are propagated in the TEM mode (Scott, 1993).

1.6.4 Reflection

When microwaves are propagating from one microwave transmission component to another, the change in electric and magnetic field configurations result in reflection of radiation at the junction between the two components (Scott, 1993). Reflection also occurs at the interface between two media having different EM properties (Michaelson and Lin, 1987). For example, every object has an impedance value which describes how that object supports an EM wave (Scott, 1993). In order to ensure complete transmission of an EM wave into an object, the impedance of the wave must be perfectly matched with the impedance of the object (Scott, 1993). If the impedances are not matched reflection will occur at the object surface. Similarly, when a wave exits an object and encounters another object, the impedances of the two objects must be perfectly matched in order to avoid reflection at the interface.

The amount of radiation reflection that occurs at an interface between two microwave transmission components can be expressed as a reflection coefficient (ρ), power reflection coefficient (ρ^2), or as a standing wave ratio (SWR) (Scott, 1993). The **reflection coefficient** is the ratio of the reflected electric field strength to the incident electric field strength at the interface (Michaelson and Lin, 1987). The ratio of the reflected power to the incident power is the **power reflection coefficient** (Scott, 1993). The reflection coefficient can be obtained by taking the square root of the power reflection coefficient (Scott, 1993).

When two waves are travelling in opposite directions in the same space, as when reflection occurs, the resulting electric field is the sum of the incident electric field and the reflected electric field (Michaelson and Lin, 1987). When the two waves are in phase with one another the fields add together resulting in a maximum electric field. When they are out of phase, the fields subtract to give a minimum electric field. The resulting peaks and troughs of electrical energy tend to stay in the same place over time if there is no disturbance, and are referred to as **standing waves** (Scott, 1993). The SWR is the ratio of $(1 + \rho)/(1 - \rho)$. The point where the maximum electric field occurs is called a "hot spot" and these have been reported to occur at the interface between bone and tissues of high water content (Johnson and Guy, 1972). Waveguides often contain standing waves due to the difference in field configurations between the waveguide and connected device. The SWR in a waveguide can be measured by inserting a probe and measuring the voltage difference between the highest and lowest points on the wave (Scott, 1993). A SWR of 1

indicates a perfect match between microwave components so there is no reflection of microwaves at the junction (Scott, 1993).

1.6.5 Attenuation

The amplitude of an EM wave attenuates (loses energy) exponentially as it passes through a lossy dielectric material (one which has energy storage capacity) (Michaelson and Lin, 1987). Therefore, with depth a wave will eventually attenuate to an arbitrarily small power. The depth at which the wave may be considered to diminish to negligible power is equivalent to 10 skin depths, where a skin depth is defined as the distance over which an EM wave attenuates to 30% of its original value (Scott, 1993). Since power is proportional to the square of the electric field, at 30% of the electric field the power has a value of $(0.3 \times E)^2$ = 10% power.

The penetration depth into different tissues varies depending on the water content of the tissue and the frequency of radiation. For high water containing tissues such as skin and muscle the penetration depth at 915 MHz and 2450 MHz microwave radiation are 3.04 cm and 1.7 cm, respectively (Johnson and Guy, 1972). These values increase to 17.7 cm and 11.2 cm, respectively, for low water containing tissues such as fat and bone (Johnson and Guy, 1972).

For attenuation to occur, energy must be converted into a different form. Usually, microwave energy is converted into heat and the amount of heat generated depends on the dielectric constant of the object. The dielectric constant is a measure of the energy storage capacity of a material (Serway, 1986).

1.6.6 Absorption

The amount of power absorbed by an animal placed in a microwave field is described by the specific absorption rate (SAR). The SAR describes the time rate of energy absorption per gram of tissue from nonionizing radiation (Michaelson and Lin, 1987). According to the International Radiational Protection Association (IRPA, 1988), the whole body average SAR should not exceed 0.4 W kg^{-1} during occupational exposure to radiofrequency energy above 10 MHz. This value is based on reviews of appropriate literature which suggest that a threshold acute exposure of 4 W kg^{-1} is sufficient to cause behavioural changes in animals (Repacholi, 1990). However, due to the possibility of adverse health effects following prolonged exposure, a 10-fold reduction in permissible SAR (i.e. 0.4 W kg^{-1}) was invoked (IRPA, 1988).

1.7 Biological effects of electromagnetic radiation

1.7.1 Introduction

The biological effects of electromagnetic radiation have been the subject of much debate over the past 20 years and continue to cause great concern for people exposed to EM energy in their daily lives. The main reason for concern are the results of some epidemiological studies which have linked EM energy exposure to the occurrence of several types of cancer (Pool, 1990). However, a review of the huge collection of research articles written on the biological effects of EM radiation reveal opposing results. On the other hand, a great deal of variation exists between research protocols which make it

difficult to compare results. For example, some researchers have used pulsed wave radiation while others have used continuous wave radiation; frequencies range from the VLF to the UHF; power levels range from $< 1 mW\ cm^{-2}$ to $> 10\ mW\ cm^{-2}$; and SARs are very difficult to assess and even more difficult to compare when different species are used. In addition to these discrepancies, there is a wide array of target areas which have been studied in an attempt to elucidate the exact level at which EM radiation is acting to achieve its biological effect. For the microwave region of the spectrum, some areas which have been studied include the central nervous system (CNS), the blood-brain barrier (BBB), the haematopoietic system, the eyes, the auditory system, endurance and performance, and behaviour. Studies investigating the effect of microwaves on behaviour alone have varied in the type of behaviour and the experimental techniques used. Furthermore, there has been much controversy surrounding the mechanism by which microwaves induce behavioural effects. Some researchers argue that the effect is strictly due to thermal stress while others insist that behavioural changes occur even in the absence of thermal stimulation.

1.7.2 Behavioural effects of microwave radiation

There have been many attempts to determine the effect of microwave radiation on behaviour in animal models. To accomplish this goal, both natural (eating, drinking, spontaneous activities) and acquired behaviours (learned responses to various stimuli) have been studied with varying results (Blackwell and Saunders, 1986). In 1975, Michaelson et al. found that rats increased their activity after exposure to 2450 MHz Mw radiation at

18 mW cm^{-2} for 45 minutes and at 36 mW cm^{-2} for only 30 minutes. Mitchell et al.(1977) also found an increase in locomotor activity in rats exposed to 2450 MHz at a dose rate of 2.3 mW g^{-1}. Similar results were reported by D'Andrea et al.(1979; 1980) in which wheel running activity by rats increased following Mw exposure at 2450 and 915 MHz, respectively. However, a greater increase (30%) was noted at 2450 MHz using a lower dose of 1.23 mW g^{-1} compared to the 25% increase by rats exposed to 915 MHz at a dose of 2.46 mW g^{-1}.

In contrast to the results of these studies, Hunt et al. (1975) found that rats exhibited a decrease in exploratory behaviour following exposure to 2450 MHz pulsed wave (PW) microwave radiation at 6.3 mW g^{-1} even after a 1 hour delay following irradiation. However, at the same dose rate, rats failed to show any deficit in swimming behaviour immediately following irradiation or 24 hours later. Only at 11 mW g^{-1} did rats show an initial decrease in swimming behaviour immediately following irradiation. In 1977, Lin et al. found that rats exposed to 918 MHz in the near field also demonstrated a decrease in performance which became more obvious with increasing power densities. Furthermore, in 1979, Gage et al. noted that rats appeared to be sleeping when irradiated with 2450 MHz Mw radiation at 15 mW cm^{-2} and an ambient temperature of 22°C. These rats assumed a more stretched out position when the ambient temperature reached 28°C. In contrast, mice tended to become more active under similar exposure conditions at 28°C. In 1994, Braithwaite et al. demonstrated that piglets displayed decreased activity level upon exposure to 2450 MHz Mw radiation at a power density of 11.2 mW cm^{-2}. Obviously, there is a great deal of variation in irradiation protocols with respect to

frequency used, power densities, SAR's, and environmental temperatures. These differences make it difficult to determine if there is a consistent pattern of behavioural effects of Mw radiation or if there is a threshold for effect. It is possible that the effect may be power density dependent as indicated by Lin et al. in 1977. This may also account for the findings of some researchers such as Roberti et al. (1975) who reported no effect of Mw radiation on spontaneous motor activity in rats exposed to 10.7 GHz continuous wave (CW), 3 GHz CW or 3 GHz PW Mw radiaton at a power density of 1 mW cm^{-2}.

The second approach used to determine if exposure to Mw radiation has an effect on behaviour involves observing its effect on conditioned responses. Once again, the studies have revealed variable results. In 1976, De Lorge demonstrated decreased response rates and an increase in latency to detection response rate in rhesus monkeys following exposure to Mw. The monkeys also showed an increase in post-reinforcement pauses from previously established stable performances on a variable interval (60 sec) schedule for food reinforcement. The threshold for the behavioural effect in this study was 72 mW cm^{-2} and the effect attenuated with repeated exposure to microwaves. In contrast, Galloway (1975) reported that rhesus monkeys exposed to 2450 MHz microwaves failed to show deficits in discriminative behaviour even at doses which produced skin burns and severe convulsions. On the other hand, Hunt et al. (1975) found that rats demonstrated an increase in omission error rate on a discrimination test following exposure to 2450 MHz PW radiation at 6.5 mW g^{-1} and a greater increase at 11 mW g^{-1}. Additional evidence of a disruption of appetitively reinforced operant behaviour was noted by Mitchell et al.(1977) who reported a deterioration of discriminative responding by rats immediately following exposure to

2450 MHz CW Mw radiation at 2.4 mW g^{-1}. However, rats used in this study showed no evidence of disruption of Sidman avoidance response. In 1975, Thomas et al. also demonstrated a decreased response rate by rats trained on a multiple fixed ratio (FR) schedule of operant conditioning upon exposure to 2860 MHz PW, 9600 MHz PW and 2450 MHz CW radiation. However, in the same study an increase in responding was noted on a more complex schedule of differential reinforcement of low rate (DRL) operant conditioning. The highest increase on the DRL schedule was seen at 20 mW cm^{-2} of pulsed 2860 MHz radiation while the greatest decrease on the FR schedule was seen at 29 mW cm^{-2} pulsed 2860 MHz.

In a different approach to studying the effect of microwaves on behaviour, Carroll et al. (1980) looked at the use of high power Mw radiation as a negative reinforcement for rats to learn an operant behaviour. In this study rats failed to learn the behaviour which would allow them to escape the negative stimulus by reducing the Mw power density from 60 mW cm^{-2} to 2 mW cm^{-2}. In another study, Braithwaite et al. (1994) trained chicks to peck a target which would turn on Mw radiation as a 'reward'. However, chicks requested fewer minutes of Mw radiation than chicks trained to peck a target for IR radiation. This difference may have been due to the greater depth of penetration by microwaves resulting in a longer lasting heating effect rather than a negative effect of microwaves since chicks showed no signs of discomfort or differential in growth.

1.7.3 Thermal vs athermal effects of Mw radiation

Most researchers agree that Mw radiation does exert some influence on behaviour in animals. However, there is disagreement concerning the method by which microwaves induce this effect. Some investigators suggest that the effect is athermal in nature while many others suggest that the effect is due to either a localized or generalized rise in temperature.

Proponents of the athermal effect of Mw radiation generally argue that the effects produced did not coincide with an increase in core body temperature. For example, Mitchell et al.(1977) reports that changes in activity level by rats exposed to 2450 MHz Mw occurred despite no indication of rectal temperature elevation. Similarly, Hunt et al. (1975) demonstrated that a decrease in exploratory behaviour by rats exposed to 2450 MHz PW radiation was not due directly to a thermal effect since there was no improvement after a 1 hour delay following irradiation.

Several suggestions have been put forth in an attempt to explain how microwaves can interact with the living organism and produce an observable effect in the absence of heat production. One such mechanism was described by Schwan in 1958 and involves the breakage of hydrogen bonds as a result of polarized side chains on macromolecules lining up with the electric field. This can result in denaturation or coagulation of proteins. A similar mechanism has been suggested by Taylor (1981) in which macromolecules may be distorted as a result of the transfer of energy from the EM field to the vibrational modes of the molecules. It is suggested that the vibration can result in enough displacement to distort the molecule into a new conformation. Another athermal effect of Mw radiation is

the auditory effect produced by pulsed waves. In a study by Frey (1962), human volunteers reported hearing clicks when exposed to PW radiation. According to Lin (1978), the clicks are produced as a result of thermally produced transient pressure waves within the head being detected by the cochlea. In 1976, Johnson et al. attributed detection of a 918 MHz PW field by rats to an auditory effect since substitution of an acoustic cue with Mws resulted in continued performance of an operant behaviour. Many other researchers have since reported auditory effects of microwaves in animal subjects (eg. Chou and Guy, 1979; Hjeresen et al., 1979; Chou et al., 1985).

Although athermal effects of microwaves have been demonstrated by several investigators, the majority of researchers support the thermal mechanism of Mw biological effects. According to Johnson and Guy (1972), microwaves generate heat through two mechanisms: ionic conduction and vibration of dipole molecules of water and proteins. Depending on the cooling capacity of the tissue, as it absorbs microwave power, the tissue heats up. In fact, since microwaves are able to penetrate beneath the skin, deep tissues and organs may be heated in this way. Servantie and Gillard (1983) suggest that this internal heating of an organism may be the method by which animals detect high level fields. Indeed there have been many reports of an increase in rectal temperature coincident with observed behaviour changes. For example, De Lorge (1978) reports that behavioral disruption in rats, squirrel monkeys and rhesus monkeys is associated with power densities that produce an increase in rectal temperatures of at least 1°C above control levels. Furthermore, Carroll et al. (1980) and Adair (1983) suggest that deeply penetrating microwaves can produce direct injury to subdermal tissues before the threshold of pain is

reached. This may explain why Galloway (1975) did not see behavioural deficits in monkeys exposed to microwaves even at doses that produced severe burns and convulsions.

Supporters of the thermal mechanism of microwaves can also argue that behavioural changes observed in the absence of increased core body temperature may also be attributed to a thermal effect. This is explained by the fact that skin and hypothalamic temperatures have a greater effect on behavioural thermoregulation than rectal temperature (Corbit, 1970). According to Corbit's adjustable set-point model of thermoregulation, a response is initiated when the hypothalamic temperature and the set-point temperature differ (i.e. an error signal is generated). This error signal can be generated by a change in either the hypothalamic temperature or the set-point temperature which is influenced by skin temperature, level of arousal, exercise, pyrogens and inputs from extra-hypothalamic core temperature receptors (Corbit, 1970).

The fact that a simple change in skin temperature can affect thermoregulatory responses is demonstrated by Brown and Brengelmann (1970) in an experiment using human volunteers. The metabolic rate of humans rose rapidly in response to a drop in water bath temperature while tympanic and rectal temperatures remained stable. Furthermore, an increase in water bath temperature following cold stress immediately resulted in a decrease in metabolic rate even though central body temperature was quite low (~36°C). Corbit (1970) speculates that the behavioral response to changes in skin temperature enable one to escape thermal stress before any change in core temperature can be affected.

Hypothalamic temperature also plays a very important role in thermoregulation and may also explain the behavioural effects of microwaves in the absence of colonic temperature changes. It has been demonstrated by Corbit (1970) that heating the anterior hypothalamus of rats while at an environmental temperature of 25°C (within the rat's thermoneutral zone) resulted in a decrease in rectal temperature and an increase in the performance of a cooling behaviour. This suggests that, although the rat's body temperature was adequate, an increase in the hypothalamic temperature is sufficient to induce behavioural thermoregulation. In addition, hypothalamic warming caused a decrease in general activity level and an increased incidence and duration of sleep. This may explain the results of many studies in which animals exposed to Mw radiation displayed reduced activity levels and the tendency to sleep (eg. De Lorge, 1976; Gage et al., 1979, Braithwaite, et al., 1994). Murgatroyd and Hardy (1970) confirmed the findings of Corbit by showing that warming the hypothalamus of rats, using surgically implanted thermodes perfused with warm water, in a hot environment resulted in increased work to obtain cool air while cooling the hypothalamus by perfusion of the thermodes with cold water, inhibited this behaviour. Baldwin and Ingram (1967) found similar effects of hypothalamic warming and cooling in pigs.

In addition to the behavioral effects of Mw radiation, other observed effects may be attributed to tissue heating. For example, several investigators have reported an increase in blood-brain barrier permeability upon exposure to microwaves. Frey et al. (1975) reported an increase in fluorescein uptake by certain areas of the rat brain following microwave exposure. Further studies by Merritt et al. (1978) and Williams et al.(1984)

confirm the results obtained by Frey et al. and submit that fluorescein content increased with increasing exposure and increasing brain temperature. A study by Moriyama et al. in 1991 substantiates the claim of a thermal effect on the BBB. In this study it was noted that extravasation of horseradish peroxidase occurred in brain tissue heated above 44.3°C for 30 min and at 42.5°C for 60 min. However, Mw irradiation failed to open the BBB when brain temperatures were sustained below 40.3°C by a cooling system.

Hypothalamic warming may also be responsible for endocrine changes reported due to Mw radiation. The neural and endocrine controls over body temperature are integrated at the hypothalamus. Therefore, it seems reasonable to suggest that hypothalamic warming will, in turn, affect the hormonal pathways which are regulated by the hypothalamus. In fact, Lotz and Michaelson (1979) found that hypophysectomized rats exposed to 2450 MHz microwaves at 60 mW cm^{-2} for 1 hour had corticosterone levels that were lower than controls while intact rats had increased levels of corticosterone in another study by Lotz and Michaelson (1978). This finding suggests that the corticosterone response to microwaves is dependent on adrenocorticotropin release by the pituitary (Lu et al., 1980). Other studies have linked changes in the endocrine system to increased colonic temperatures. For example, Lotz and Michaelson (1978) found a correlation between increased colonic temperature and elevations of plasma corticosterone in rats exposed to 2450 MHz Mw radiation. In addition, Lu et al. (1985) reported that decreased levels of serum thyroid stimulating hormone (TSH) corresponded to increased colonic temperatures in rats exposed to 2450 MHz Mw radiation. In spite of these changes in endocrine hormones, Michaelson and Lin (1987) suggests that they do not necessarily indicate a

pathological effect since such changes may occur in an attempt to maintain homeostasis within the organism.

1.8 Objectives of the research

There has been extensive research on the biological and behavioural effects of Mw radiation on living organisms. However, thus far, no studies have been undertaken to quantify the effect of 915 MHz Mw radiation on activity level in weaner pigs. Furthermore, there has been no attempt to establish a link between Mw-induced alteration in activity level with circulating levels of the sleep-inducing hormone, melatonin. The goal of this research is to extend the current knowledge of Mw bioeffects by providing data on these above-mentioned unexplored areas. The working hypothesis with which this research has been undertaken is that Mw radiation causes a decrease in activity level among weaner pigs which is more pronounced with increasing Mw power level.

2.0 ACTIVITY LEVEL AND PERFORMANCE OF WEANER PIGS EXPOSED TO 915 MHz MICROWAVE RADIATION

2.1 Summary

Microwave radiation (915 MHz) is considered as a possible source of supplementary heat for early weaned pigs. To determine the behavioral effect of this form of heat on weaner pigs, 4 trials were carried out in which 64 pigs were exposed to either IR or one of two levels of microwaves, Mw1 (11.4 mW cm^{-2}) or Mw2 (6.1 mW cm^{-2}), each following a 4 day adaptation period. Pigs exposed to both Mw treatments displayed greater ($P < 0.05$) daily percent resting time compared with IR-exposed pigs (86.0 ± 0.40 vs 82.3 ± 0.63 vs 79.4 ± 0.66% (mean ± SEM) for Mw1, Mw2, and IR, respectively). The increase in resting time was greatest after the first day of treatment and gradually returned to pre-treatment levels over the course of the 3 week experiment. The treatment effect over time was also significant ($P < 0.05$) between IR and both Mw treatments and indicated similar patterns of resting time for the Mw treatments which were different from the pattern displayed by IR treated animals. The pattern of resting behaviour for the IR group remained relatively unchanged throughout the experiment. Microwave exposure did not have an effect ($P > 0.05$) on average daily gain (0.32 ± 0.02 vs 0.28 ± 0.02 vs 0.30 ± 0.03 kg day^{-1} (mean ± SEM) for IR, Mw1 and Mw2, respectively). The results indicate that 915 MHz Mw causes a power level-dependent decrease in activity in weaner pigs. However, Mw exposure does not significantly affect performance in weaner pigs.

2.2 Introduction

At weaning, piglets have very little body fat (~17%) (Mount, 1968) or hair and therefore require supplemental heat for optimum performance if room temperatures are below 26°C (English et al., 1977). Conventional methods of heating weaner rooms such as heating lamps, underfloor heating, gas heaters or radiators, although effective, are costly and present ventilation problems (Whittemore, 1993).

Recently, Mw has been considered as a possible source of supplementary heating for young livestock such as pigs and chicks (Braithwaite et al., 1994). Microwaves cause polar molecules, such as water, to vibrate. The resulting friction raises the internal temperature of the animal (Copson, 1975). Shanawany (1990) demonstrated that Mw can increase body temperature linearly with exposure time. The penetration depth of Mw is determined by its frequency and the water content of the tissue or object irradiated. Penetration depth decreases with increasing frequency and with increasing water content. The penetration depth of 915 MHz Mw is approximately 3 cm into muscle due to the high water content, while it can penetrate up to 17.7 cm into bone (Johnson and Guy, 1972). The Mw method of heat transfer does not directly affect the temperature of the air or other dry materials in the animal's environment. As a result, given the body water content, Mw energy is an efficient source of heat that allows the animal to be maintained in a well ventilated environment. The study by Braithwaite et al. (1994) involving the use of 2450 MHz Mw as a heat source revealed that Mw in newly weaned pigs did not adversely affect body weight, feed:gain ratio, or age at first estrous. However, lower activity level was observed among piglets using a Mw heated deck when compared to those in an IR heated deck (Braithwaite et al., 1992). Before implementing

Mw technology in production units the physiological basis of this type of behavioral effect should be examined. Prior to carrying out such physiological studies however, a quantification of the behavioral effect of Mw is necessary. The objective of this study, therefore, was to compare the activity level and performance of weaner pigs exposed to 915 MHz Mw or IR.

2.3 Materials and Methods

2.3.1 Treatments

The study was conducted in four replicates, each consisting of three treatments: infrared exposure at 500 W (n = 32), Mw exposure at 204 W (Mw1) (11.4 mW cm^{-2}) (n = 16), and microwave exposure at 109 W (Mw2) (6.1 mW cm^{-2}) (n = 16). The specific protocol was approved by the University Animal Care Committee and the management of the animals followed the recommendations of the Canadian Council on Animal Care (Olfert et al., 1993).

2.3.2 Microwave Equipment (Figure 1)

Four cages of stainless steel (151 x 122 x 81 cm) with one solid steel side, three wire mesh sides (6 x 6 mm holes and 1 mm thick wire) and rubberized expanded metal grid flooring (Tenderfoot ™)(2.5 x 1.0 cm holes) were used to house the piglets. All four cages were placed inside an environmentally controlled room (see section 2.3.4). The floors of the cages were 47 cm above the room floor. Two cages were assigned to the IR treatment and one to each Mw treatment. The source of the Mw energy was a generator with a maximum

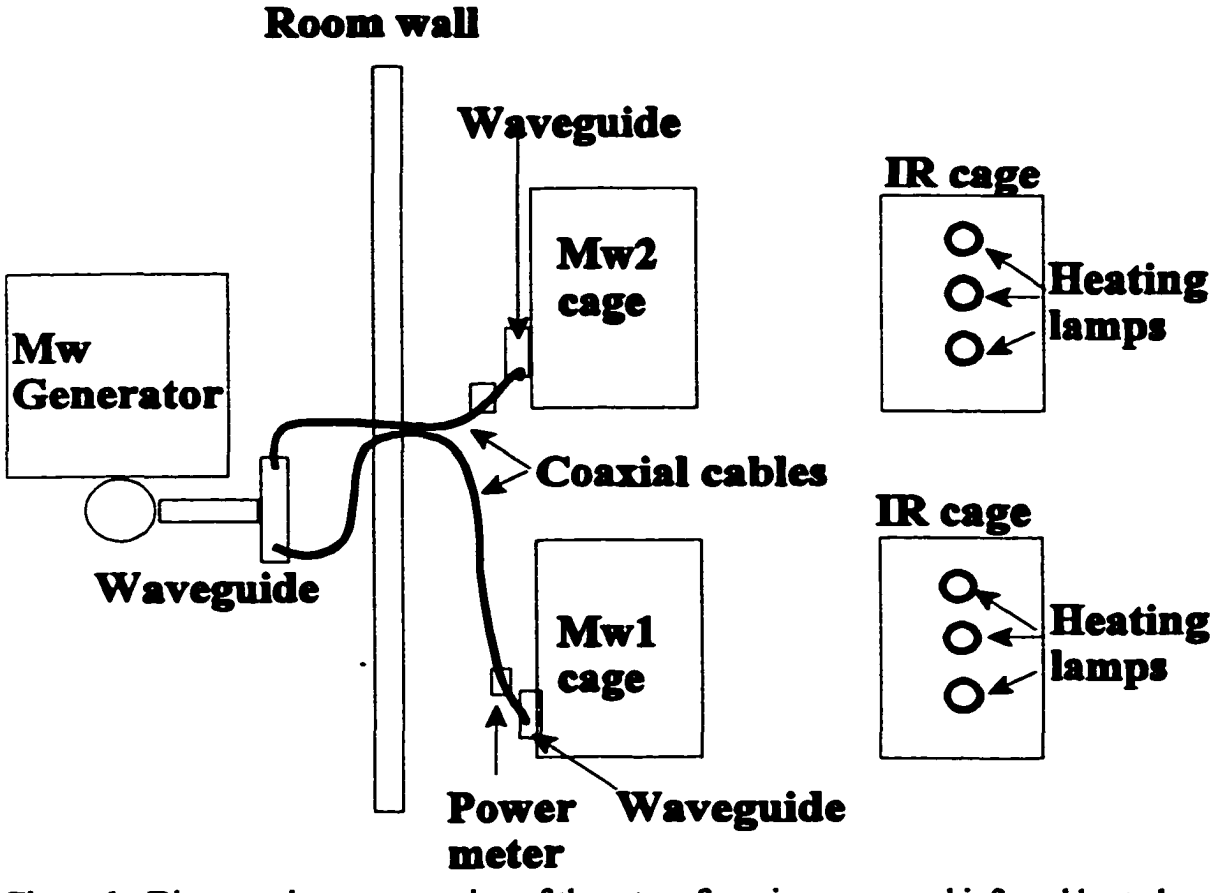


Figure 1. Diagramatic representation of the set up for microwave and infrared heated cages.

output of 4 kW of 915 MHz Mw. The generator which had adjustable power output was acquired from D'Ossone Canada Ltd (Charlottetown, PEI). Microwaves were delivered to the two Mw cages via a stainless steel 'T'-shaped waveguide. The stem and the top of the waveguide measured 34 and 69 cm long, respectively and the cross section measured 25.4 x 12.7 cm. The waveguide was equipped with two antennae located at opposite ends of the top portion of the waveguide. Two 300 ohm coaxial cables (2.6 and 2.3 m) were connected to the antennae and carried the Mw energy to a second 28 cm long waveguide located on the back wall of each weaner deck. The forward power in each cage was monitored using a Bird RF directional thruline wattmeter (Model 43, Cleveland, OH), each placed between two segments of the coaxial cables at the entrance of each waveguide. The waveguide opening at the back wall of the cages was protected by a 4 mm thick Mw-transparent teflon cover (33.0 x 20.3 cm). Each of the Mw cages was equipped with two safety switches which served to deactivate the magnetron when one of the lids was not properly closed. Microwave cages were checked frequently for leakage using a Mw survey meter, model HI 1600 (Holaday Industries Inc., Eden Prairie, MN). The IR cages were heated by two 250 W heat lamps which were placed into 25.4 cm diameter holes in the cage covers and guarded with a metal mesh.

2.3.3 Animals

For each of the 4 replicates 16 piglets (3-4 weeks of age) from four litters (2 males and 2 females from each litter) were marked with a permanent hair dye for identification. One member of each litter was randomly allocated to each of the four cages. As a control for sex

differences, 2 males and 2 females were assigned to each cage. This arrangement resulted in a total of 32 piglets for the IR treatment and 16 piglets for each Mw treatment. Food and water were provided *ad libitum* using commercial, galvanized steel feeders and nipple-type drinkers which were properly grounded to the exposure cage. Pigs were fed a commercial piglet starter diet containing 18% protein throughout the trial. Weights of the animals were recorded at the beginning of the trial and on a weekly basis. The average initial weight of the pigs was 6.46 ± 1.22 kg (mean ± SD). All animals were allowed to socialize for 4 days as an adaptation period which permitted the establishment of the social hierarchy within each cage. For environmental enrichment, each group of pigs was supplied with 1/4 of a rubber tire with the wire removed.

2.3.4 Environmental Conditions

Room temperature was monitored and kept at 23.3 ± 0.9°C (mean ± SD) during the four days of adaptation and was reduced to 16.8 ± 0.6°C (mean ± SD) for the remaining 20 days. This reduction in room temperature was necessary to prevent overheating of animals while the Mw and IR lamps were on. Following the adaptation period animals were supplemented with continuous exposure to Mw or to the IR lamps. Humidity levels were monitored and maintained at an average of 48.7 ± 0.5% (mean ± SD) throughout the experiment. Animals were kept on a 12h:12h light:dark schedule throughout the trial in which the lights went on at 0630 h and off at 1830 h. Light intensities during the daylight period were 106.7 lx, 104.9 lx, 63.66 lx, and 52.9 lx for Cages 1, 2, 3, and 4, respectively. Light intensities during the night time were 68.2 lx, 45.7 lx, 9.0 lx, and 13.5 lx for cages

1, 2, 3, and 4, respectively where cages 1 and 2 received IR treatments, cage 3 received Mw1 treatment and cage 4 received Mw2 treatment. Prior to the onset of treatment, light intensities during the daylight hours were 33.2 lx, 52.9 lx, 51.1 lx, and 34.1 lx for cages 1, 2, 3, and 4 respectively. During the night time in the pretreatment period, light intensities were 11.7 lx, 11.2 lx, 11.7 lx and 8.5 lx for cages 1, 2, 3, and 4, respectively. Light intensities for each cage were measured using a Gossen Panlux Electronic 2 light meter (Berlin, Germany) and are the means of 6 recordings made in the 6 planes of an imaginary cube located at the center of each cage and approximately at the pigs' eye level. During the dark period 2 flourescent emergency lights located in opposite corners of the room remained on for the duration of the experiment for video recording purposes.

2.3.5 Video Equipment

Pig behaviour was recorded continuously over a 24 day period using four Panasonic video cameras (model WV-BL 200) connected to a Panasonic time lapse video cassette recorder (model AG-6040) and a Panasonic sequential switcher (model WJ-521 altered to provide a maximum of 1.5 min switching time). For each cage pig behaviour was recorded for 1.5 minutes before switching to the next cage resulting in a total of 15 minutes viewing time per hour for each cage.

2.3.6 Behavioral Observations

Pig behaviour was recorded at the onset of each frame and was coded 0 for inactive and 1 for active. A score of 0 was recorded when pigs were sitting or laying down. All other

behaviour was recorded as active. The total resting time was calculated and expressed as a daily percentage for analysis.

2.3.7 Temperature Measurements

Throughout the experimental period, skin temperatures (ST) of pigs were taken every second day using an Omega infrared thermometer (model 0S71) and were recorded as an average of 5 areas: head, shoulders, middle back, lower back, and rump. Cage temperatures (CT) were measured using Luxtron fluoroptic temperature probes (Model 750, Mountain View, CA) which were held in place with Silly Putty® and protected with a wire mesh screen. Recordings were made every 5 minutes by an Epson LX 800 dot matrix portable printer (model OS71-PRT modified to a serial printer). Room temperature (RT), relative humidity (RH) and forward microwave power were recorded daily.

2.3.8 Statistical Analyses

Percent resting time was analysed using a one way repeated measures Analysis of Variance. A separate analysis of variance was carried out on each pair of treatments and were broken into pretreatment (the adaptation period) and experimental periods. The Wilk's Lambda test statistic was generated and used to determine if there was a Mw effect on the patterns of resting behaviour. The General Linear Models procedure in the Statistical Analysis Software version 6.04 (SAS Institute Inc., Cary, NC, 1985) package was used.

To detect Mw effect on weight gain, an analysis of covariance using initial weight as a covariate, was performed. Correlation analyses were carried out to detect any possible

relationships between daily percent resting time, microwave power level, RT, CT, ST, and humidity level.

2.4 Results

2.4.1 Activity Level

Results of the analysis for activity level revealed that the pattern of resting behaviour displayed by IR treated pigs was significantly different ($P < 0.05$) from those of the two Mw treated groups (Table I). This difference was evident in both the pre-treatment (adaptation) period and the experimental period. A comparison between each Mw treatment, however, indicated that similar resting patterns were observed between these groups. The daily percent resting time over the 24 day period for each treatment graphically demonstrates the similar patterns between each Mw group as well as the difference between these groups and the IR treated group (Figure 2). The daily percent resting times for the Mw treated pigs during the experimental period were consistently higher than those of the IR treated pigs although not always significant. Each Mw treated group demonstrated a sudden increase in resting behaviour on day 5, at the onset of the treatment. This peak was followed by a gradual decline in resting behaviour over the next 20 days. The IR treated pigs, however, showed no change in resting behaviour over the entire 24 day period. Statistical analysis comparing percent resting time by day indicated that resting time for the animals within the Mw2 group had returned to a level equal to that observed among animals in the IR group by day 17. Resting time for the pigs exposed to the Mw1 treatment, however, remained higher for the

Table I. Wilk's Lambda *P* values for pretreatment and experimental analyses of variance testing pattern differences in daily percent resting time between weaner pigs exposed to Mw1 (11.4 mW cm^{-2})(n=16), Mw2 (6.1 mW cm^{-2})(n=16) and IR (500 W) (n=32)

	P values	
Treatments	Pretreatment	Experimental
IR[a] vs Mw1[b]	0.0086[d]	0.0009[d]
IR vs Mw2[c]	0.0105[d]	0.0399[d]
Mw1 vs Mw2	0.8206	0.1226

[a] Infrared radiation
[b] Microwave radiation at 11.4 mW cm^{-2}
[c] Microwave radiation at 6.1 mW cm^{-2}
[d] Significant at the 0.05 level.

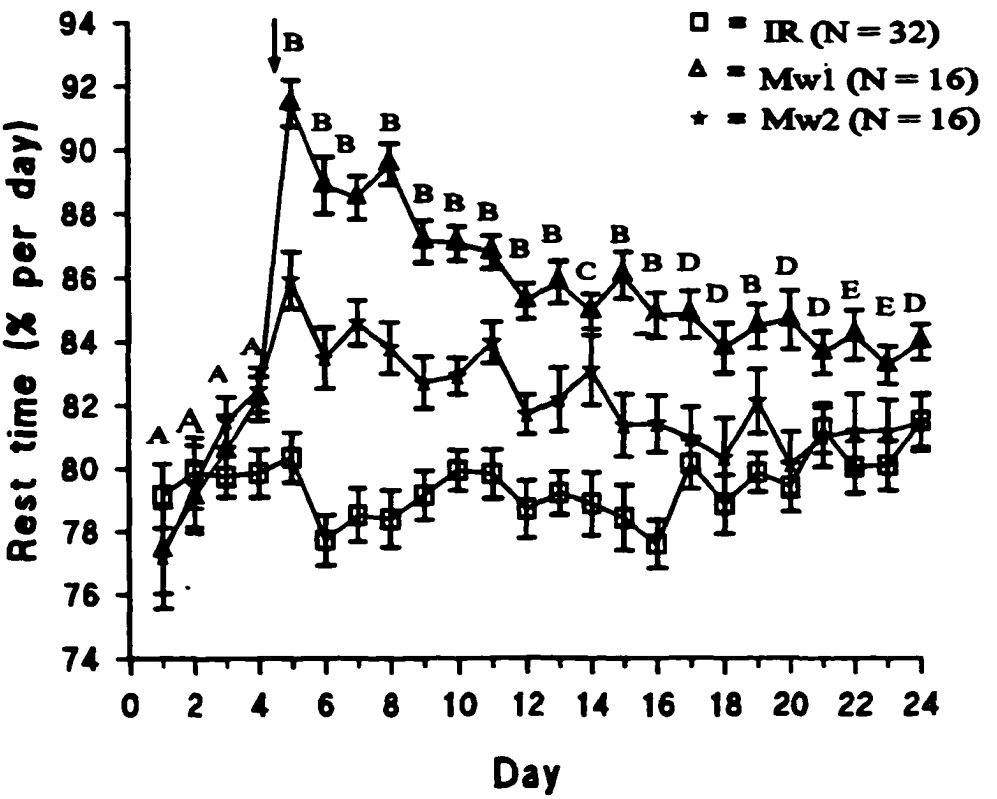


Figure 2. Mean (± SEM) percentage resting times per day for IR- (500 W), Mw1- (11.4 mW cm^{-2}), and Mw2- (6.1 mW cm^{-2}) treated pigs. Arrow indicates beginning of treatments, and data points represent average over 24-h period. Letters above each time point represent the following: A = No significant difference between all three treatments; B = All three treatments significantly different; C = No significant difference between Mw1 and Mw2 only; D = No significant difference between Mw2 and IR only; E = No significant difference between Mw1 and Mw2 or between Mw2 and IR.

remainder of the experiment ($P < 0.05$). The mean percent resting time for the IR group during the experimental period was 79.4 ± 0.66% (mean ± SEM) while that for the Mw2 group was 82.3 ± 0.63% and for the Mw1 group it was 86.0 ± 0.40%.

2.4.2 Performance

The results of the analysis of covariance on average daily gain revealed that Mw at either 6.1 mW cm^{-2} or 11.4 mW cm^{-2} had no significant effect ($P = 0.39$) on weight gain in weaner pigs (Table II). The values obtained for the average daily gain of pigs exposed to Mw1, Mw2, and IR, respectively were 0.276 ± 0.02 kg, 0.294 ± 0.03 kg, and 0.321 ± 0.02 kg (mean ± SEM). The initial weights of individual pigs were significantly different ($P < 0.05$) but this variable was accounted for in the analysis as a covariate and the values obtained have been adjusted accordingly.

2.4.3 Correlations

There was a high positive correlation between daily power level and daily percent resting time (Table III). This relationship is clearly depicted in Figure 3 which shows a low percent resting time at the lower power level and a higher percent resting time at the higher power level. Small but positive correlations were also detected between RT and daily percent resting time ($r=0.11$; $P < 0.05$), and between daily average ST and daily percent resting time ($r=-0.03$; $P < 0.05$) (Figure 4). No relationships were observed between daily CT and resting time, daily RT and daily CT or daily humidity level and resting time ($P > 0.05$).

Table II. Average body weights and standard deviations per treatment for weaner pigs exposed to IR (500W)(n = 32), Mw at 11.4 mW cm^{-2} (Mw1)(n= 16) or Mw at 6.1 mW cm^{-2} (Mw2)(N=16)

Treatment	Average body weight (kg) ± SD[a]				
	Day 0	Day 7	Day 14	Day 21	Day 25
IR	6.4 ± 1.4	7.7 ± 1.8	9.8 ± 2.5	12.5 ± 3.2	14.3 ± 3.9
Mw1	6.5 ± 1.0	7.7 ± 1.4	9.5 ± 1.7	11.9 ± 2.1	13.5 ± 2.6
Mw2	6.6 ± 1.2	7.9 ± 1.6	9.9 ± 2.2	12.2 ± 3.2	14.0 ± 3.7

[a]Standard deviation.

Table III. Pearson Correlation Coefficients and *P* values for comparisons between pig resting time, microwave power level, RT[a], CT[b], ST[c] and RH[d]

Variables	Sample size (N)	Correlation coefficient	*P* value
Daily power level vs daily % rest time	150	0.47	0.05
Daily power level vs daily % rest time (Mw1) (11.4 mW cm^{-2})	75	0.01	0.96
Daily power level vs daily % rest time (Mw2) (6.1 mW cm^{-2})	75	-0.11	0.35
Avg. daily CT vs daily % rest time	44	0.11	0.47
Daily RT vs avg. daily CT	44	-0.03	0.83
Daily RT vs daily % rest time	324	0.11	0.04[e]
Daily avg. ST vs daily % rest time	156	0.18	0.03[e]
Daily humidity vs daily % rest time	324	-0.05	0.30

[a] Room temperature
[b] Cage temperature
[c] Skin temperature
[d] Relative humidity
[e] Significant at $P = 0.05$

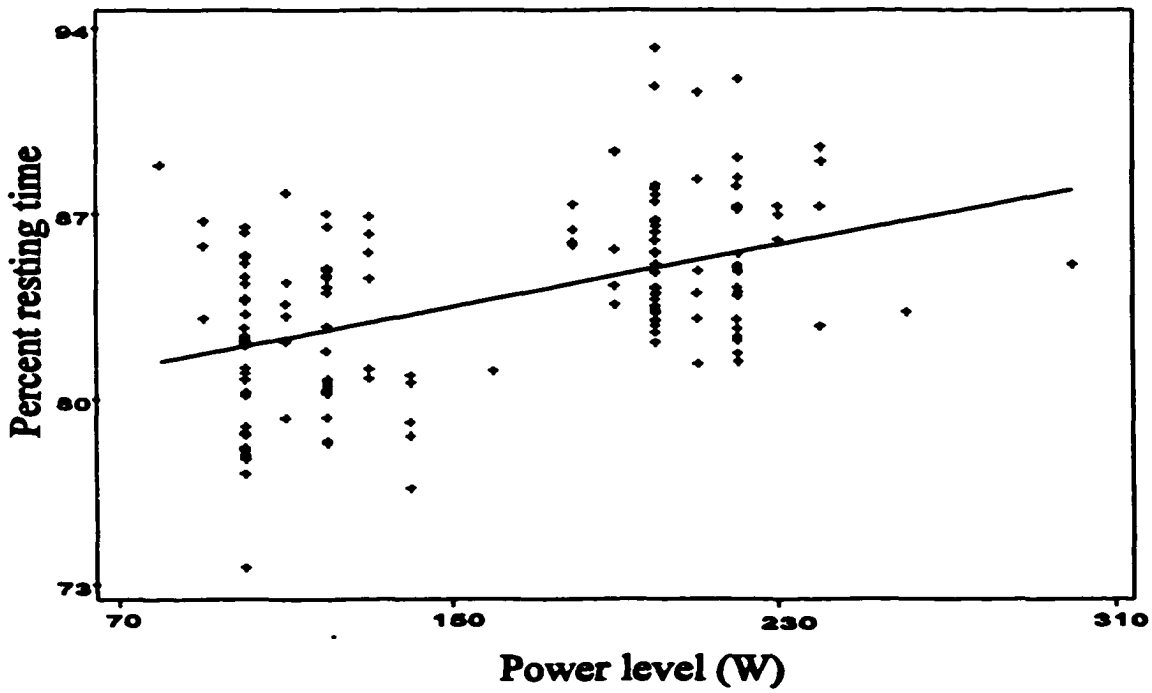


Figure 3. Effect of Mw power level (W) (x) on daily percentage resting time of weaner pigs exposed to 915 MHz Mw at 11.4 mW cm^{-2} (Mw1), 6.1 mW cm^{-2} (Mw2) and IR at 500 W ($Y = 79.1 + 0.03\ x$; $r^2 = 0.22$).

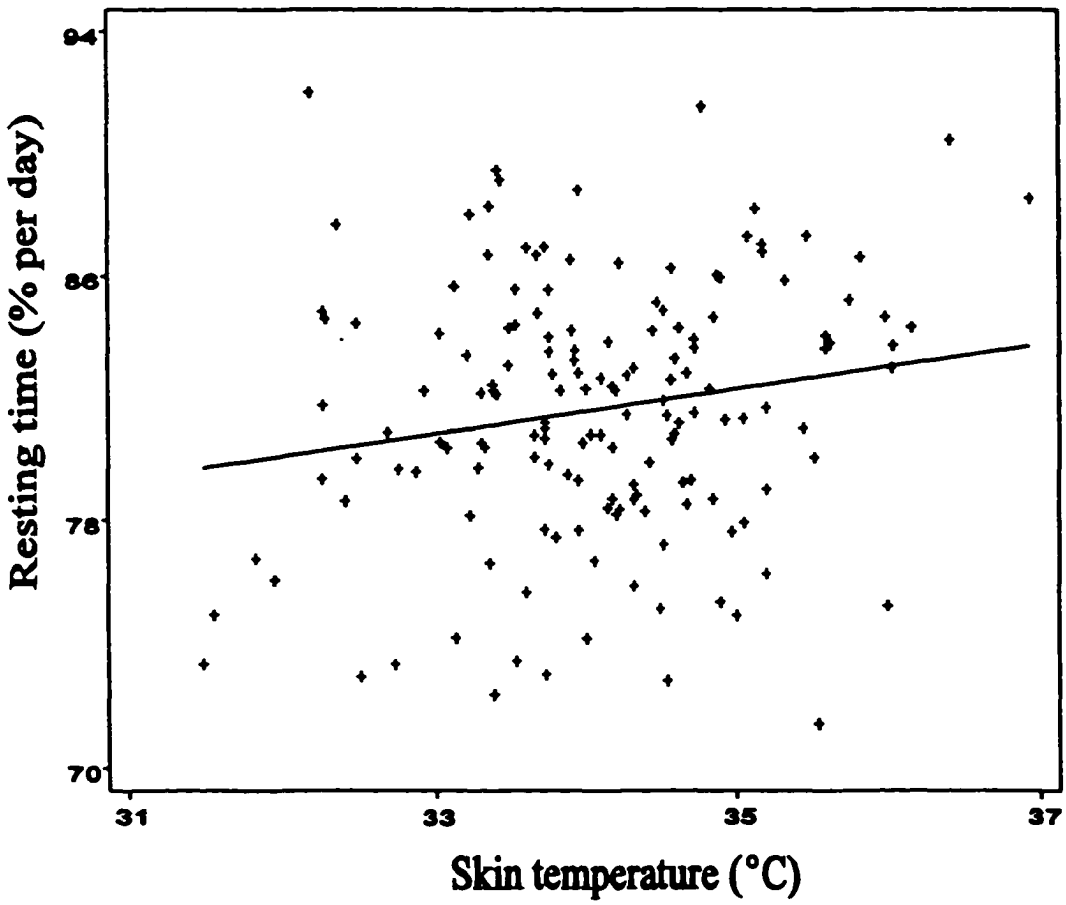


Figure 4. The effect of daily average ST (°C)(x) on daily percentage resting time of weaner pigs exposed to 915 MHz Mw at 11.4 mW cm^{-2} (Mw1), 61 mW cm^{-2} (Mw2) and IR at 500 W (Y = 56.8 + 0.73 x; r^2 =0.03) (N = 156).

2.5 Discussion

This study, in which activity level of weaner pigs exposed to 915 MHz Mw was quantified, confirms the observation by Braithwaite et al. (1992) that Mw exposure results in a reduced activity level in weaner pigs compared to IR exposure. The magnitude of this difference in activity is dependent upon the Mw power level as demonstrated by the correlation between Mw power level and daily percent resting time. Pigs that were exposed to 11.4 mW cm^{-2} spent 3.7% more time resting than those exposed to 6.1 mW cm^{-2} and 6.6% more time resting than IR exposed pigs. This suggests that the greater the power of radiation absorbed by the animal, the greater the effect on the behaviour of the animal. The maximal and minimal power levels required to trigger this response are not yet established and most likely are outside the range used in this study. These findings coincide with previous findings by Hunt et al. (1975) in which rats exposed to 2450 MHz pulsed wave microwave radiation at 6.3 mW g^{-1} demonstrated decreased levels of exploratory behaviour and reduced swimming speed. Swimming performance was more profoundly affected after exposure to 11 mW g^{-1} than after 6.3 mW g^{-1}.

The observed decline in percent time spent in resting behaviour over time indicates that an adaptation to the Mw is occurring. An adaptation to the radiation is the most likely explanation for this attenuated effect over time because the first drop in resting behaviour occurs just 24 hrs after the increase in resting behaviour. If the reduced effect were due to the increase in body weight with time the reduction would not be seen until several days had passed. Similar findings of an adaptation to Mw has been reported by De Lorge (1976) in his work on behavioral effects of Mw on rhesus monkeys. The study demonstrated that upon

exposure to 2450 MHz Mw rhesus monkeys exhibited reduced lever press responses at 72 mW cm^{-2} and this effect declined with repeated exposures over a 5 day period. A similar adaptation was observed in rectal temperature responses to Mw over the same period. The reduction and eventual disappearance of effect in this study suggests that in an attempt to maintain homeostasis the initial response of the animals to Mw exposure is behavioral until the body can compensate physiologically. Further research into the physiological effects of Mw may lead to a better understanding of possible mechanisms of action. Servantie and Gillard (1983) have documented a variety of behavioral changes as a result of Mw exposure. Some alterations resulting from modifications in membrane permeability to calcium as a consequence of Mw exposure have also been reported (Adey, 1980).

The different patterns of resting behaviour between the three treatment groups during the pretreatment period is difficult to explain at this time. The sharp rise in resting time on day 5, at the onset of the treatment, by the Mw treated animals demonstrates a treatment induced effect rather than a continuation of the pretreatment pattern.

Pigs in a hot environment with no place to wallow will thermoregulate by increasing the surface area that is in contact with the cooler floor or walls, increasing the respiration rate, and decreasing food consumption (Mount, 1968). Throughout the experiment pigs showed no visible signs of thermoregulatory behaviour in the form of panting or rapid breathing. Pigs in the Mw decks, however, sometimes slept in fully recumbent positions and were often spread around the cage. Such postures may indicate that the Mw exposed pigs were attempting to dissipate heat through conduction and avoiding contact with other pigs. On the other hand, they may simply have been adopting the relaxed postures of thermally comfortable

animals (Mount, 1968). The positive correlation detected between daily percent resting time and ST also suggests that the Mw effect on activity level may be temperature related. Many thermoregulatory processes are triggered by thermal receptors in the skin, which results in behavioral (Corbit, 1970) and physiological changes taking place before a rise in body core temperature has occurred (Brown and Brengelmann, 1970). On the other hand, this correlation is extremely weak as indicated by the low correlation coefficient and apparent random distribution of points in figure 4 and, therefore, little emphasis should be placed upon it. Similarly, a weak correlation between RT and percent resting time suggests a possible thermal effect of microwaves but, once again, the correlation coefficient is extremely low and therefore, the finding of statistical significance is unreliable.The amount of energy absorbed by animals in the Mw treatment may be more than that required to maintain homeothermic conditions as the effective environmental temperature may be much higher than anticipated.

Pigs in the IR decks were most often observed huddling beneath the heat lamps. This huddling response may be an indication of the limited capacity of IR lamps to heat the surrounding environment resulting in the need for the animal to be directly beneath the source to maximize its effectiveness. On the other hand, the huddling may be an attempt to minimize heat loss through conduction thus maximizing the benefit of the IR lamps. Mount (1968) suggested that pigs will not huddle when in a thermally comfortable environment but will do so when the temperature drops below thermal neutrality. This observation indicates that the IR exposed animals are attempting to thermoregulate behaviorally thus reducing the thermal losses to the environment.

The behaviour of Mw pigs during the active periods was consistent with that of the IR pigs suggesting that such behaviour is normal under the given experimental conditions. Normal behaviour consisted of playing with the tire, investigating their surroundings, romping around as well as eating and drinking. No unusual or obvious aberrant behaviour was observed in the Mw exposed animals in comparison to the IR treated animals.

Agonistic behaviours such as head butting, ear biting and aggressive pushing were only observed during the adaptation period and indicate an attempt to establish a social hierarchy (Hafez and Signoret, 1969). Once the social order has been determined most disturbances of an individual by a lower rank pig are settled with simple eye contact (McBride et al., 1964). Such subtle agonistic interactions are not distinguishable through the video recording system and therefore none were noted during the experimental period.

Several neurotransmitters have been studied in association with sleep. Of these, serotonin and acetylcholine have been found to play important roles in the sleep process (Webb and Dube, 1981). Jouvet (1988) suggested that serotonin may act as a neurohormone which plays a role in the onset of slow wave sleep through its action on the anterior hypothalamus/basal forebrain. Acetylcholine has been known to induce sleep when applied to many areas of the central nervous system (Hernández Peón, 1965). It is possible that microwave radiation may be inducing sleep in weaner pigs via one of these neurochemicals, either by affecting its production, release, or uptake.

The hypothalamus also has important implications in the sleep-wake cycle. The suprachiasmatic nucleus has been found to have many functions including a timing center for the circadian rhythms of pineal serotonin N-acetyltransferase activity (Moore and Klein,

1974), brain temperature (Stephan and Nuñez, 1977), heart rate (Saleh and Winget, 1977), and several other biological rhythms. The role of the suprachiasmatic nucleus in the circadian rhythm of serotonin N-acetyltransferase activity is important in the production of melatonin which is also associated with sleep onset (Anton-Tay et al., 1971). Microwave radiation could be exerting its effect via direct action on the hypothalamus. There are many possibilities for the mechanism in which Mw induces sleep in weaner pigs. Although there was no statistically significant difference in ADG between Mw- and IR-eposed pigs, the actual values for ADG were lower for Mw-exposed pigs. This may be a reflection of a potential reduction in food intake as a result of inactivity. The implications of a possible reduction in food intake are both beneficial and detrimental. Reduced activity may mean that the pigs are expending less energy and therefore, can put extra energy into growth. On the other hand, if they are not consuming more food than is required for maintenance at the level of activity displayed by these pigs, it could mean reduced growth rates. However, no conclusions can be made about this at this time as feed consumption was not measured.

The performance results of Mw treated pigs were consistent with those obtained by Braithwaite et al. (1994) indicating that Mw does not affect weight gain in weaner pigs. The results obtained by Morrison et al. (1987) demonstrated that Mw reduced the feed:gain ratio of male broiler chicks. However, chicks in that study were exposed to intermittent 2450 MHz Mw in contrast to the continuous wave 915 MHz radiation used in this study. The absence of a Mw effect on weight gain suggests that these animals were not experiencing serious thermal stress. Furthermore, the behavioral effect of Mw is insufficient to result in a change in performance. Pigs maintained in a hot environment reduce their food consumption and

therefore would not gain as much as those which were maintained in a thermoneutral or cooler environment (Mount, 1968).

Results of the correlation analyses further emphasize the power level-dependent effect of Mw on activity in weaner pigs. The high correlation (0.47) between Mw power level and resting time is strong evidence that the effect of Mw on activity level is determined by the power of radiation to which the animals are exposed. The correlation between ST and resting time suggests a temperature related effect of the Mw. However, the correlation was small (correlation coefficient of 0.18) indicating that the relationship is very weak and may only be coincidental. Furthermore, no relationship was detected between CT and resting time which indicates that the ambient temperature is not interacting with the treatment to effect a change in the activity level of the pigs. Therefore, any thermal effect on activity must be the result of the Mw power level. More work should be done to determine the optimal level of Mw which will result in obtaining the maximum benefit of this technology.

2.6 Conclusions

Exposure to Mw at 915 MHz causes an initial decrease in activity level among newly weaned pigs when compared to IR exposed pigs. This effect is dependent upon the Mw power level inducing a greater decrease in activity at higher power densities than at lower power densities. Furthermore, the effect of Mw on activity diminishes with continued exposure in such a way that activity returns to basal levels after three weeks of exposure. Performance of pigs exposed to Mw is similar to that of pigs exposed to IR.

3.0 PLASMA MELATONIN, CORTISOL AND GLUCOSE CONCENTRATIONS IN WEANER PIGS EXPOSED TO 915 MHz MICROWAVE RADIATION

3.1 Summary

A study was undertaken to determine the physiological basis of the documented reduction in activity level of weaner pigs exposed to 915 MHz microwave radiation. Pigs were exposed to one of two different microwave power levels (Mw1 at 6.9 mW cm^{-2} or Mw2 at 3.8 mW cm^{-2}) or infrared radiation (IR) at 250 W. Blood samples were obtained at frequent intervals over a 24 hour period for plasma melatonin, cortisol and glucose concentration determination. Circulating plasma melatonin concentrations were highest ($P < 0.05$) for Mw1-treated pigs and were followed by Mw2-treated pigs (16.26 $\pm$ 0.97 pg mL^{-1}, 13.20 $\pm$ 1.05 pg mL^{-1}, and 8.68 $\pm$ 0.85 pg mL^{-1} for Mw1, Mw2 and IR, respectively) (mean $\pm$ SEM). The 24-h profile of plasma melatonin was inconsistent among pigs. Approximately 18% of the pigs demonstrated low day time and high night time melatonin concentrations. Mean plasma cortisol concentrations were similar for each group of pigs (1.96 $\pm$ 0.12 μg dL^{-1}, 1.96 $\pm$ 0.12 μg dL^{-1}, and 2.40 $\pm$ 0.12 μg dL^{-1} for Mw1, Mw2 and IR, respectively). Mean plasma glucose concentration was highest ($P < 0.05$) for Mw1-treated pigs at 93.44 $\pm$ 0.87 mg dL^{-1}. However, glucose concentrations were similar for Mw2 and IR treated pigs (85.43 $\pm$ 0.87 mg dL^{-1} vs 82.76 $\pm$ 1.10 mg dL^{-1} for Mw2 and IR, respectively). These results indicate that exposure to 915 MHz microwave radiation results in an intensity-dependent increase in plasma melatonin and glucose concentrations but does not initiate a stress response in weaner pigs at power levels

$\leq$ 6.9 mW cm^{-2}. Potential mechanisms by which microwaves induce increased melatonin production are discussed.

3.2 Introduction

The application of microwave radiation as an alternative source of heat for livestock has been investigated by various researchers (Morrison et al., 1986; 1987; Shanawany, 1990; Braithwaite et al., 1992; 1994; Acorn, 1996; Foote et al., 1996). Several of these studies have reported obvious reductions in activity level in pigs exposed to microwave radiation without significant effects on performance (Braithwaite et al., 1992; 1994; Foote et al., 1996). Mount (1968) reported that pigs will reduce their food consumption in a hot environment. Therefore, if the reduction in activity is due to overheating, one would expect to see depressed growth rates in microwave exposed pigs. These reports of unaffected growth rates in microwave exposed pigs warrant further investigation in an attempt to elucidate the mechanism by which microwave radiation suppresses activity in newly weaned pigs.

In recent years, much has been learned about the role of melatonin in the sleep-wake cycle (Marczynski et al., 1964; Barchas et al., 1967; Vollrath et al., 1980). Melatonin is a hormone produced mainly within the pineal gland of animals and humans. In most species studied, plasma melatonin levels exhibit a circadian rhythm in which peak levels are associated with the scotophase of the light-dark cycle and low levels are associated with the photophase (Lynch, 1971; Hedlund et al., 1977; Coetzee et al., 1989; Wilson and Gordon, 1989). However, the melatonin rhythm can undergo a phase shift in

nightshift workers and in subjects exposed to artificial lighting during the night and darkness during the day (Czeisler et al., 1986; 1989; Shanahan and Czeisler, 1991). In pigs, however, it appears that the nocturnal surge of melatonin occurs only in some animals and only during a 12h:12h light:dark schedule (McConnell and Ellendorff, 1987; Minton and Cash, 1990; Griffith and Minton, 1992). It has also been demonstrated that administration of exogenous melatonin will induce sleep in cats (Marczynski et al.,1964), chicks (Hishikawa et al., 1969) and humans (Antón-Tay et al., 1971; Cramer et al., 1974; Vollrath et al., 1980).

Although there are no known studies on the effect of high freqency electromagnetic fields (the microwave frequncy range) on melatonin secretion, there is documented information on the effects of low frequency electric and magnetic fields on melatonin secretion in humans (Reiter, 1993), rats (Wilson et al., 1981; Kato et al., 1993; 1994a; 1994b), the Djungarian hamster (Yellon and Gottfried, 1992) and nonhuman primates (Rogers, 1995). Most of these reports indicate a reduction in melatonin secretion upon exposure to electric and magnetic field frequencies of 50 Hz (Kato et al., 1993 and 1994b) and 60 Hz (Wilson et al., 1981; Yellon and Gottfried, 1992; Rogers, 1995). In a previous study by Foote et al. (1996) a reduced activity level was noted in weaner pigs exposed to 915 MHz Mw. In light of the sleep-inducing effect of exogenously administered melatonin it was hypothesized that this higher frequency of electromagnetic radiation stimulates melatonin secretion resulting in decreased activity in weaner pigs.

Stress in animals has been known to result in stimulation of the hypothalamo-pituitary-adrenal (HPA) axis with subsequent elevations in circulating corticosteroid

concentrations (Curtis, 1983). Glucocorticoids, in turn, stimulate gluconeogenesis resulting in elevated plasma glucose concentrations. Research into the effects of Mw radiation on living animals has utilized these parameters to determine if Mw exposure elicits a stress response in the subjects studied. For example, in 1973, Parker demonstrated that rats exposed to 2450 MHz Mw radiation at 15 mW cm^{-2} for 16 h showed no elevation in plasma corticosteroid concentration. On the other hand, Lotz and Michaelson (1978) found that rats exposed to 2450 MHz Mw radiation for 30 or 60 min at 50 or 60 mW cm^{-2} or for 120 min at 20, 30 or 40 mW cm^{-2} showed elevated plasma corticosteroid levels which coincided with elevations in colonic temperature. Similar results were found by Lu et al. (1977) however, in this study, temperature was increased at times without a corresponding increase in serum corticosteroid concentration. These results suggest that a stress response by animals exposed to Mw radiation may be due to heat stress. Therefore, it is hypothesized that Mw radiation at low power density (i.e. < 10 mw cm^{-2}) which does not produce elevations in body temperature will not stimulate the HPA axis.

The purpose of this study is to determine if plasma melatonin, cortisol and glucose concentrations are altered in pigs exposed to microwave radiation at 915 MHz compared with pigs exposed to infrared radiation and if this treatment induces a stress response. It is hypothesized that Mw radiation induces an increase in circulating melatonin levels in weaner pigs without a corresponding increase in cortisol or glucose.

3.3 Materials and methods

3.3.1 Experimental design

The experiment was carried out in two replicates. Twelve crossbred pigs were used for each replicate. Within each replicate animals were assigned to one of three treatments: IR exposure at 250 W (industry control)(n = 12), microwave exposure at 128.6 ± 17.2 W (mean ± SD) (Mw1) (6.9 mW cm^{-2})(n = 6), and Mw exposure at 70.7 ± 15.4 W (Mw2) (3.8 mW cm^{-2})(n = 6). The power levels of microwave exposure used in this experiment were different from the previous experiment (see section 2.3.1). These power levels are averages taken over the entire experimental period. The target power level for the Mw1 treatment was 109 W which was the power level used in the previous experiment for the Mw2 treatment. This power level was chosen because the behavioural modification was noted at 109 W in the previous experiment and was considered sufficient to demonstrate an alteration in plasma melatonin concentration if it existed. The lower power level of 70.7 W was the average power level obtained when a target of 75 W was chosen. This power level was chosen to determine if a behavioural effect as well as altered melatonin concentration were present at power levels lower than that used in the previous experiment. All pigs were obtained from a certified minimal disease operation at 3.5 (replicate 1) to 4.5 weeks of age (replicate 2). For each of the replicates, 4 pigs each were taken from 3 litters and were allocated to 4 cages (2 IR, 1 Mw1 and 1 Mw2) such that there were 3 pigs per cage and each litter was represented in each cage. Also, the animals were distributed so that each cage had representatives of both sexes but were not balanced

due to the uneven number of animals per cage. Pigs were individually housed within each cage but were able to see and hear penmates. The protocol was approved by the University Animal Care Committee and follows the guidelines of the Canadian Council on Animal Care (Olfert et al., 1993).

3.3.2 Equipment

Animals were housed in the weaner decks as described in section 2.3.2 with a few variations. Microwave transparent plexiglas dividers (122 cm x 80 cm x 0.6 cm) were installed to prevent physical interaction between the animals to avoid entanglement and destruction of the catheters. This partitioning of the weaner deck meant that 3 plastic commercial feeders (Agri-plastics) were required. In addition, a small hole (1.3 cm diameter), which did not permit microwave leakage, was cut in the cover above each compartment to exteriorize the catheters. Each animal was provided with its own 1/4 of a rubber tire as environmental enrichment as well as a rubber dog toy.

The microwave equipment as described in section 2.3.2 was used for this trial. Infrared cages were equipped with three 250 W IR lamps, one located directly above each of the compartments .

The video equipment described in section 2.3.5 was also used in this trial but the time period of continuous recording was shortened to 6 days starting when pigs were assigned to respective cages.

3.3.3 Environmental conditions

Animals were assigned to one of 4 weaner decks upon arrival and given 2 days to acclimatize to the new environment. On the third day, cannulation surgeries were performed on all pigs as described in section 3.3.4, followed by a 3-day recovery period during which animals received analgesics (butorphanol, 0.5 mg kg^{-1}, orally) ($T_{½}$ = 3-4 h in humans) twice per day. The analgesics were given via syringe rather than in the feed to ensure that each animal recieved the proper dose. On the seventh day, animals were exposed to either IR or Mw radiation and blood samples were collected over a 24 hr period as per the schedule described in section 3.3.6. Room temperature during the adaptation and recovery periods was monitored and maintained at 23.5 ± 2.0° C (mean ± SD). During the experimental period the temperature was reduced to 17.6 ± 0.7° C (mean ± SD). Humidity level was monitored and maintained at 63.3 ± 8.7% (mean ± SD) throughout the entire trial.

Fluorescent lights were maintained on a 12h:12h light:dark schedule with two corner ceiling lights remaining on at all times for continuous 24 hr video recording. Lights went on at 0630 h and off at 1830 h. Light intensities within each cage were as stated in section 2.3.4.

3.3.4 Cannulation surgery

The evening before surgery food was removed at 2000 h and water was removed the following morning at 0700 h. The pigs were premedicated with 2.6 mg kg^{-1} azaperone (Janssen, Toronto, Ontario) ($t_{½}$ ~ 3.2 h) IM and general anaesthesia was induced with 6.4

mg kg^{-1} ketamine (Vetapharm Canada Inc., London, Ontario) ($t_{½}$ ~ 2 h) IM and 0.03 mg kg^{-1} atropine sulfate (MTC Pharmaceuticals, Cambridge, Ontario) ($t_{½}$ ~ 2 h) IM. Halothane (MTC Pharmaceuticals, Cambridge, Ontario) at 1.5% with oxygen (flow rate of 2 L min^{-1}) was used for maintenance of a surgical plane of anaesthesia. Each animal was shaved along the ventral aspect of the neck and a nonsterile scrub was performed using 3 alternations of 70% isopropyl alcohol (Ingram and Bell Medical, Don Mills, Ontario) and 4% chlorhexadine solution (Steri-Stat®)(Ingram and Bell Medical, Don Mills, Ontario) followed by a sterile scrub using the same solutions. Animals receiving a radiotransmitter were also shaved and scrubbed in a similar manner on the ventral abdomen.

Cannulation surgery involved placement of a sterilized catheter made from medical grade vinyl tubing (Bolab®, size 6; internal diameter 0.86 mm, outer diameter 1.27 mm)(Duval Plastics and Engineering, Auburn, Australia) in the jugular vein of each pig. Each catheter was equipped with a vinyl cuff to be positioned at the entrance to the vein to prevent the catheter from sliding into or out of the vein. The catheter was placed by making a vertical skin incision in the ventral neck to the right of midline and exposing the right jugular vein following blunt dissection of the subcutaneous tissue and fat. Two Bulldog clamps were placed on the jugular vein ~ 3.5 cm apart and a v-shaped notch was cut in the vein using Metzenbaum scissors. The catheter was advanced approximately 10 cm into the vein and tied in place with 3-0 Prolene® (Davis and Geck, Bedford, N.S.) suture material just proximal to the vinyl cuff. This was followed by ligation of the jugular vein immediately cranial to the point of catheter entry and incorporating the catheter just distal to the vinyl cuff. The catheter was then carried subcutaneously using a 12 G, 4 inch

needle, up to the back of the neck where it exited the skin. The tubing was glued in position at the skin opening with Super Glue™ (Home Hardware Stores, Ltd., St. Jacobs, Ontario). The skin incision was closed with 2-0 Maxon® (Davis and Geck, Bedford, N.S.) suture material. The catheter was then carried to the middle of the animal's back and secured in several places with skin staples. The animal was wrapped from neck to back with Vetwrap™ (3M Animal Care Products, St. Paul, MN) to prevent damage to the catheter and to protect the incision site. A piece of sterilized braided nylon fishing line (~ 2.9 m long) was tied to the animal's back by threading it through a 12 G needle inserted through the skin. The catheter was taped to the fishing line which was carried up through a hole in the center of the cage cover, over the top of 2 parallel aluminum pipes (45 cm apart) near the ceiling, and down in front of the cage where a weight (~ 20 g) was tied to the end. This counter balance setup kept the catheter out of the animals' reach while allowing them to move freely within the cage. The fishing line held the weight so that there was no strain on the catheter.

Catheter patency was maintained by a constant flow of sterile saline (0.9% sodium chloride solution (Baxter Corporation, Toronto, Ontario) with 30 IU mL^{-1} heparin (Leo Laboratories Canada, Ltd, Ajax, Ontario)) via an intravenous solution set delivering fluid at a rate of 60 drops mL^{-1}. The IV drip was set at ~ 1 drop per 10 s for a total of 1 mL per 10 min. The catheter was connected to the IV set with a three-way nylon stopcock (American Pharmaseal Co., Valencia, Ca) to allow blood collection without removal of the IV set.

3.3.5 Radiotransmitter implantation

During the cannulation surgery, one pig from one of the microwave treatments in each replicate was randomly chosen to receive a Lotek radiotransmitter (Aurora, Ont.) that was placed in the abdominal cavity through an incision along the ventral midline of the abdomen. The incision was closed with 2-0 Maxon® (Davis and Geck, Bedford, N.S.). The radiotransmitter sent out signals at a frequency of 151.013 MHz that were detected by a Lotek telemetry receiver (model SRX 400, Aurora, Ont.) which converted the signal to a temperature reading. This temperature was used as an indicator of core body temperature. The results obtained regarding core body temperature are described in Appendix A.

3.3.6 Blood collection

Sample collection consisted of withdrawing heparinized saline from the catheter until pure blood was obtained, switching syringes to collect 2 mL of blood and changing syringes again to inject 5 mL of heparinized saline (30 IU mL^{-1}). Samples were collected in Vacutainer™ brand (Becton Dickinson, Franklin Lakes, NJ) evacuated blood collection tubes (72 USP units of sodium heparin) and placed in the refrigerator prior to centrifuging which was done within 24 h of collection. The samples were centrifuged for 5 min at 2500 g and the plasma was transferred to 2 Eppendorf (Brinkman Instruments Co., Westbury, NY) disposable micro-centrifuge tubes (1.5 mL, polypropylene). Plasma samples were stored at -25° C for subsequent biochemical analysis.

Blood was collected from each pig according to the following schedule:

First sample 1/2 h prior to Mw or IR exposure

Second sample at the start of the treatment

Samples every 1/2 h for 2 h

Samples every hour for 3 h

Samples every 3 h for remaining 18 h

This sampling schedule provided a total of 15 samples per pig over a 24 h period (or ~5% of blood volume)(Reece, 1991). However, some samples were not obtained due to failure of catheters to remain operational. Additional parameters recorded at each sample time included room temperature, room humidity level, microwave power delivered to each microwave cage, and body core temperature of the pig carrying the radiotransmitter.

3.3.7 Biochemical analyses

Plasma samples were analysed for melatonin, cortisol, and glucose concentrations. Melatonin concentrations were determined using the Melatonin Direct ^{125}I-RIA kit (Elias USA, Inc., Osceola, WI) and were prepared and analysed in duplicate. The Melatonin radioimmunoassay is a three-step procedure in which melatonin concentrations in plasma or serum are quantitatively determined with a sensitivity of 1.5 pg mL^{-1}. In the first step melatonin is enzymatically released from its binding proteins in the sample. This is followed by addition of an assay buffer, melatonin antibody and ^{125}I-labelled melatonin. In the final step, a precipitating antibody is added and the sample is centrifuged (3250 g,

25 min, at 4°C), decanted and counted using a gamma counter (Riastar 5400, Packard, Donner's Grove, Ill.). Reported crossreactivity studies indicate that the melatonin ^{125}I RIA shows only slight cross reactivity with N-Acetylserotonin (0.8%), 5-Methoxytryptophol (0.7%), and 5-Methoxytryptamine (0.08%). Less than 0.01% crossreactivity was reported with 6-Methoxytryptamine, 5-Methoxyindole-3-acetic acid, serotonin, DL-Tryptophan, DL-5-Methoxytryptophan, and 5-hydoxy-L-tryptophan. The melatonin assays conducted in our lab had intraassay coefficients of variation of 17.3%, 30.8% and 20.6% for assays 1, 2 and 3, respectively as determined using a pooled plasma sample from one pig. The interassay coefficient of variation was 28.3%. Values that were off the standard curve (too low) were considered unreliable and were not included in the analysis. This meant that 57 single samples and 36 duplicate samples were discarded. Also, melatonin values that were extremely high and believed to be inaccurate due to the short half life of melatonin in plasma (~45 minutes), were not included in the analysis (6 samples). It should also be noted that the standards used in this kit were human serum samples.

Cortisol concentrations were determined using the Coat-A-Count® Cortisol radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA.). Each sample was prepared and analysed in duplicate. The sensitivity of the assay was ~0.2μg dL^{-1}. The reported crossreactivity associated with this assay indicated that there is substantial crossreactivity with prednisolone (76%), slight crossreactivity with 11-deoxycortisol (11.4%) and very slight crossreactivity with prednisone (2.4%). However, crossreactivity with other naturally occurring steroids or therapeutic drugs was less than 1%. Cortisol assays conducted in our lab had intraassay coefficients of variation of 12.3%, 7.0%, and

14.4% for assays 1, 2, and 3, respectively as determined using a pooled plasma sample from one pig. The interassay coefficient of variation was 18.6%. Once again, the standards used in this assay were human serum samples.

Glucose concentrations were determined using the Beckmann Glucose Analyser 2 and Beckmann reagents, also as duplicate samples.

3.3.8 Behavioural observations

Pig behaviour was recorded as described in section 2.3.6. However, behaviour was only recorded for 1 week. Behaviour data was used to determine if there was any association between plasma melatonin concentration and sleep onset.

3.3.9 Statistical analyses

A general linear model procedure of SAS Institute, Inc. (version 7, Cary, NC, 1997) was used to analyse melatonin, cortisol and glucose data. For each data set the residuals (y-axis) were plotted against the predicted value as determined by the model (x-axis). A Shapiro-Wilk test for normality was carried out using SAS. These tests for normality revealed that the melatonin and cortisol data sets were not normally distributed ($P < 0.05$). Therefore a $\log_e$ transformation was performed to obtain a normal distribution ($P > 0.05$). However, results are presented using original values for both melatonin and cortisol data. The glucose data was determined to be normally distributed in its original form and did not require a $\log_e$ transformation.

A general linear model procedure of SAS Institute, Inc. (1997) was also carried out on the baseline (0800 h samples) melatonin, cortisol and glucose data in order to verify that baseline values were similar between treatment groups prior to the onset of the treatments.

Correlation analyses were conducted using Statistix version 4.0 (Analytical Software, 1992) to determine if there was a relationship between percent resting time and plasma melatonin concentration.

3.4 Results

3.4.1 Melatonin data

Differences in plasma melatonin concentrations ($P < 0.05$) during the experimental period were detected between all three treatment groups (Mw1 vs IR, Mw2 vs IR, and Mw1 vs Mw2) (Table I). Mean plasma melatonin concentration for Mw1-treated animals was greater than that for Mw2-treated animals which, in turn, were greater than that for IR-treated animals (16.26 ± 0.97 pg mL^{-1}; 13.20 ± 1.05 pg mL^{-1}; and 8.68 ± 0.85 pg mL^{-1}, for animals in Mw1, Mw2 and IR treatments, respectively)(mean ± SEM). However, there were no differences ($P > 0.05$) in plasma melatonin concentrations for Mw1-, Mw2- or IR-treated pigs prior to the start of the treatments (Table I). The mean melatonin concentrations for each group at the 0800 sampling time were 9.16 ± 2.76 pg mL^{-1}, 9.10 ± 3.04 pg mL^{-1} and 7.56 ± 2.57 pg mL^{-1} for Mw1, Mw2 and IR groups, respectively (mean ± SEM). In addition, there was no significant difference ($P > 0.05$) in melatonin concentrations between replicates for the 0800 sampling time. However, the mean melatonin concentration for replicate 1 was

Table L *P* values for pretreatment and experimental analyses of variance testing differences in mean plasma melatonin concentrations (pg mL^{-1}) of weaner pigs exposed to 915 MHz Mw at 6.9 mW cm^{-2} (Mw1) (n = 6), 3.8 mWA cm^{-2} (Mw2) (n = 6) and IR at 500 W (n = 11).

	P values	
Treatments	Pretreatment	Experimental
Mw1 vs. IR	0.8324	0.0001[a]
Mw2 vs. IR	0.1730	0.0001[a]
Mw1 vs. Mw2	0.2590	0.0092[a]

[a] Significant at $P = 0.05$

Table II. *P* values for repeated measures analysis of variance testing differences in mean plasma concentrations of melatonin (pg mL^{-1}), cortisol (μg dL^{-1}) and glucose (mg dL^{-1}) in weaner pigs exposed to 915 MHz Mw at 6.9 mW cm^{-2} (Mw1) (n = 6), 3.8 mW cm^{-2} (Mw2) (n = 6) and IR at 500 W (n = 11).

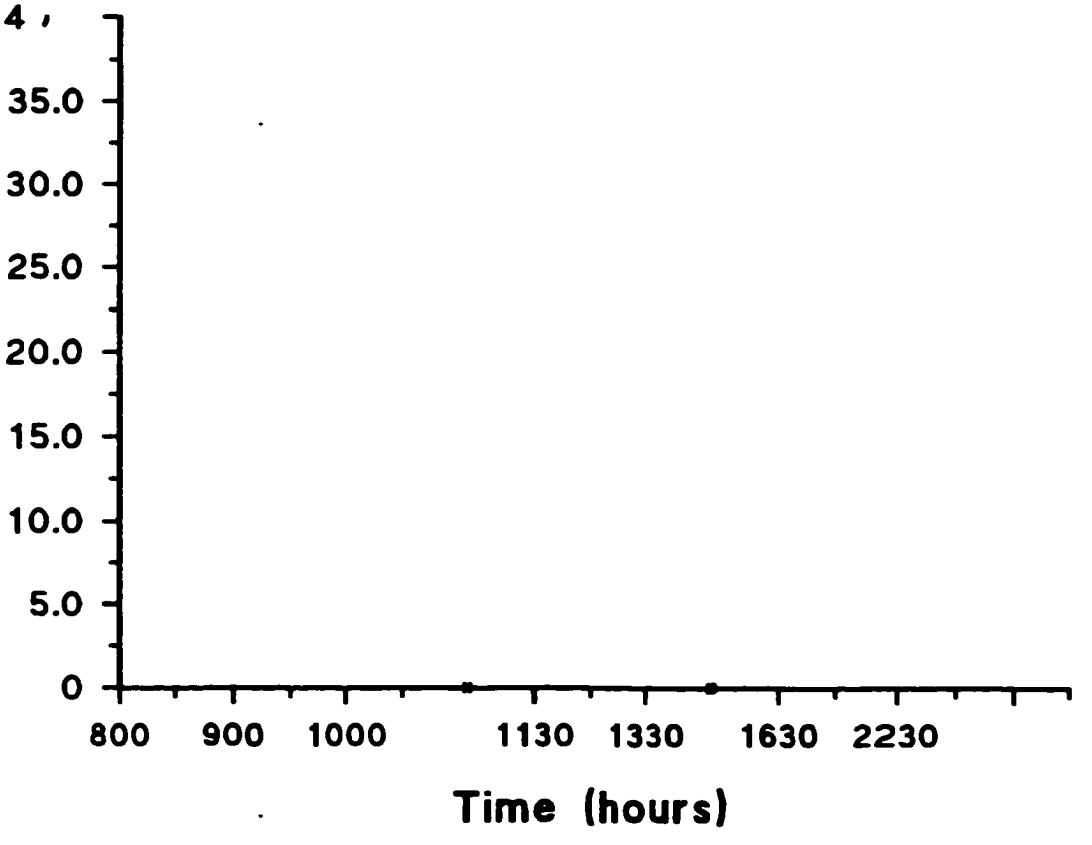
	P values		
	Melatonin	Cortisol	Glucose
Treatment	0.0001[a]	0.0908	0.0001[a]
Replicate[b]	0.0001[a]	0.0001[a]	0.0001[a]
Pig within treatment	0.0001[a]	0.0001[a]	0.0001[a]
Time	0.0001[a]	0.0001[a]	0.2023
Treatment x time	0.7004	0.3627	0.8376

[a] Significant at $P = 0.05$

[b] Type I sum of squares *P* value used

higher than that for replicate 2 (10.72 ± 2.30 pg mL^{-1} vs. 6.49 ± 2.26 pg mL^{-1} for replicates 1 and 2, respectively). Significant ($P < 0.05$) replicate, pig within treatment and time effects were also noted (Table II). However, there was no significant treatment x time interaction ($P > 0.05$) indicating that there was no difference in the patterns of melatonin concentrations between treatment groups over time.

The patterns of melatonin concentration over the 24 hour period for each treatment group (both replicates combined) are presented in Figure 1. Mean plasma melatonin concentrations remain at fairly stable low levels from 0800 h to 1230 h for all three treatment groups (range = 9.01 - 14.23; 8.68 - 12.59; 5.93 - 7.86 pg mL^{-1} for Mw1, Mw2 and IR treatments, respectively). Between 1230 h and 0730 h of the next day, the plasma melatonin profile for the Mw1 group is characterized by 2 peaks; the first peak occurred at 1330 h (26.28 pg mL^{-1}) and the second, smaller peak occurred at 0100 h (21.83 pg mL^{-1}). Plasma melatonin concentration declined to a level that is comparable to the previous morning by 0730 h. For the Mw2 group, the plasma melatonin profile is again characterized by two peaks. However, this time the first peak is delayed until 1930 h and is comparable in magnitude to the second peak for the Mw1 group at 21.76 pg mL^{-1}. The second peak for the Mw2 group occurred at 0730 h (22.67 pg mL^{-1}) at which time blood sampling was discontinued making it difficult to tell whether this is the actual peak or if the next value would have been higher still. Unlike the Mw-treated groups, the plasma melatonin profile for the IR-treated group had only one peak which occurred at


4 ,
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0
800
900
1000
1130
1330
1630
2230
Time (hours)

1930 h and is comparable in magnitude to the peaks seen in the Mw2 group at 21.43 pg mL^{-1}. By 0730 h plasma melatonin concentration for the IR group returned to a level (7.99 pg mL^{-1}) equivalent to the previous morning's values. This figure also shows the difference in melatonin levels between treatments, with melatonin concentrations for Mw1-treated animals maintaining the highest values and those for the IR-treated animals maintaining the lowest values. Furthermore, plasma melatonin secretion shows a daily rhythm with low levels during the morning hours (0800 - 1230 h) and higher values during the afternoon and night time hours (1330 h - 0730 h) which was present in all three treatment groups.

Figures 2 and 3 illustrate the differences between replicates for plasma melatonin concentration. When these figures are compared it is obvious that plasma melatonin concentrations were higher during replicate 1 for all three treatment groups than they were in replicate 2. A second notable difference between replicates is that the peak in plasma melatonin at 0730 h for the Mw2 group is only evident in replicate 1. During replicate 2, melatonin concentration declines to the previous morning's level at 0730 h for the Mw2 group. Finally, the plasma melatonin concentration for Mw1-treated pigs displayed an obvious increase immediately following the onset of the Mw treatment in replicate 1 which is not evident in replicate 2 and was not seen in the Mw2-treated pigs.

Another noteworthy finding is that not all pigs showed the daily pattern of melatonin secretion in which melatonin values are elevated during the dark hours and decreased during the light hours as evidenced by the graphs of the individual animals' melatonin concentrations over the 24 h period (data not shown). Several of the pigs showed melatonin concentrations during the night time hours that were similar to those

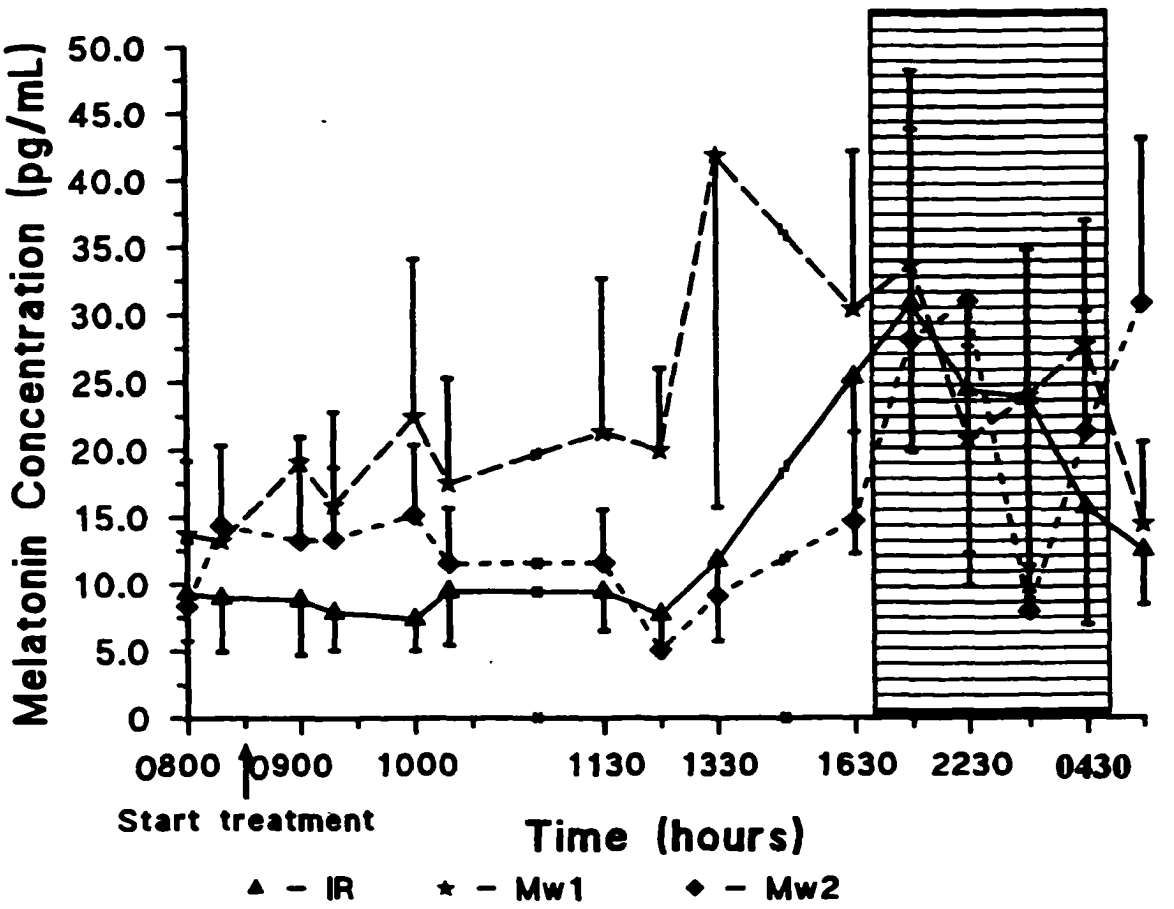


Figure 2. Mean plasma melatonin concentrations (pg mL^{-1}) ± SEM of weaner pigs exposed to IR at 500 W (n = 11), Mw at 6.9 mW cm^{-2} (Mw1) (n = 6) and 3.8 mW cm^{-2} (Mw2) (n = 6) for replicate 1 with outliers deleted. Shaded area represents the dark phase of the sampling period.

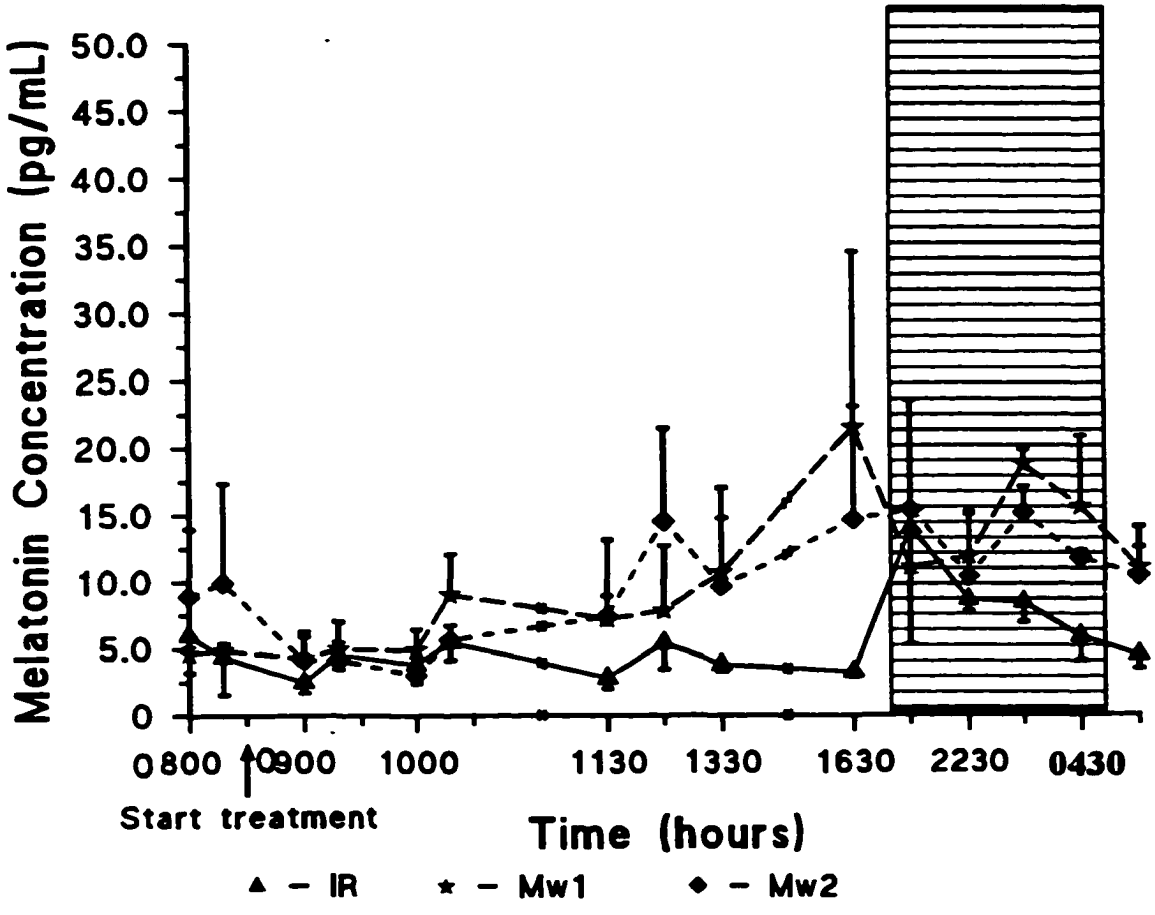


Figure 3. Mean plasma melatonin concentrations (pg ml^{-1}) of weaner pigs exposed to IR at 500 W (n = 11), Mw at 6.9 mW cm^{-2} (Mw1) (n = 6) and 3.8 mW cm^{-2} (Mw2) (n = 6) for rep 2 with outliers deleted. Shaded area represents the dark phase of the sampling period.

during the day time hours in the IR, Mw1 and Mw2 treated groups. Other pigs displayed night time melatonin concentrations that were two and three times greater than the morning concentrations. This difference between individual animals is reflected in the analysis of variance which indicated that there was a significant difference ($P < 0.01$) between pigs within treatment for melatonin concentrations (Table I).

3.4.2. Cortisol data

There was no difference ($P > 0.05$) in cortisol concentrations between the three treatments during the pretreatment or the experimental sampling times (Table III). Mean cortisol concentrations for IR-, Mw1- and Mw2-treated pigs were 2.40 $\pm$ 0.12 μg dL^{-1}, 1.96 $\pm$ 0.12μg dL^{-1}, and 1.96 $\pm$ 0.12 μg dL^{-1}, respectively. There was also no significant treatment x time interaction for plasma cortisol concentrations. Plasma cortisol values over a 24 hour period (Figure 4) exhibit similar daily patterns between treatment groups with the following exceptions: 1) at 1130 h both the IR- and Mw2-treated groups showed a peak in plasma cortisol while the Mw1-treated group showed a decline and 2) at 0730 h both the IR- and Mw1-treated groups showed a peak in plasma cortisol while the Mw2-treated group showed a decline. Otherwise, all three groups followed a similar pattern of cortisol concentrations characterized by a rapid decline between 0800 h and 0900 h, a peak at 1130 h for the IR and Mw2 treated groups which rapidly returned to low levels between 1130 h and 1230 h and a final peak at 0730 h the next morning for IR and Mw1 groups. There was little fluctuation in plasma cortisol values for most of the 24 h period in any of the treatment

Table III. *P* values for pretreatment and experimental analyses of variance testing differences in mean plasma cortisol concentrations ($\mu g\ dL^{-1}$) of weaner pigs exposed to IR at 500 W (n = 11), Mw at 6.9 mW cm^{-2} (Mw1) (n = 6) and 3.8 mW cm^{-2} (Mw2) (n = 6).

	P values	
Treatments	Pretreatment	Experimental
Mw1[a] vs. IR[b]	0.2551	0.0596
Mw2[c] vs. IR	0.6210	0.0770
Mw1 vs. Mw2	0.5551	0.9157

[a] Microwave radiation at 6.9 mW cm^{-2}

[b] Infrared radiation at 250 W

[c] Microwave radiation at 3.8 mW cm^{-2}

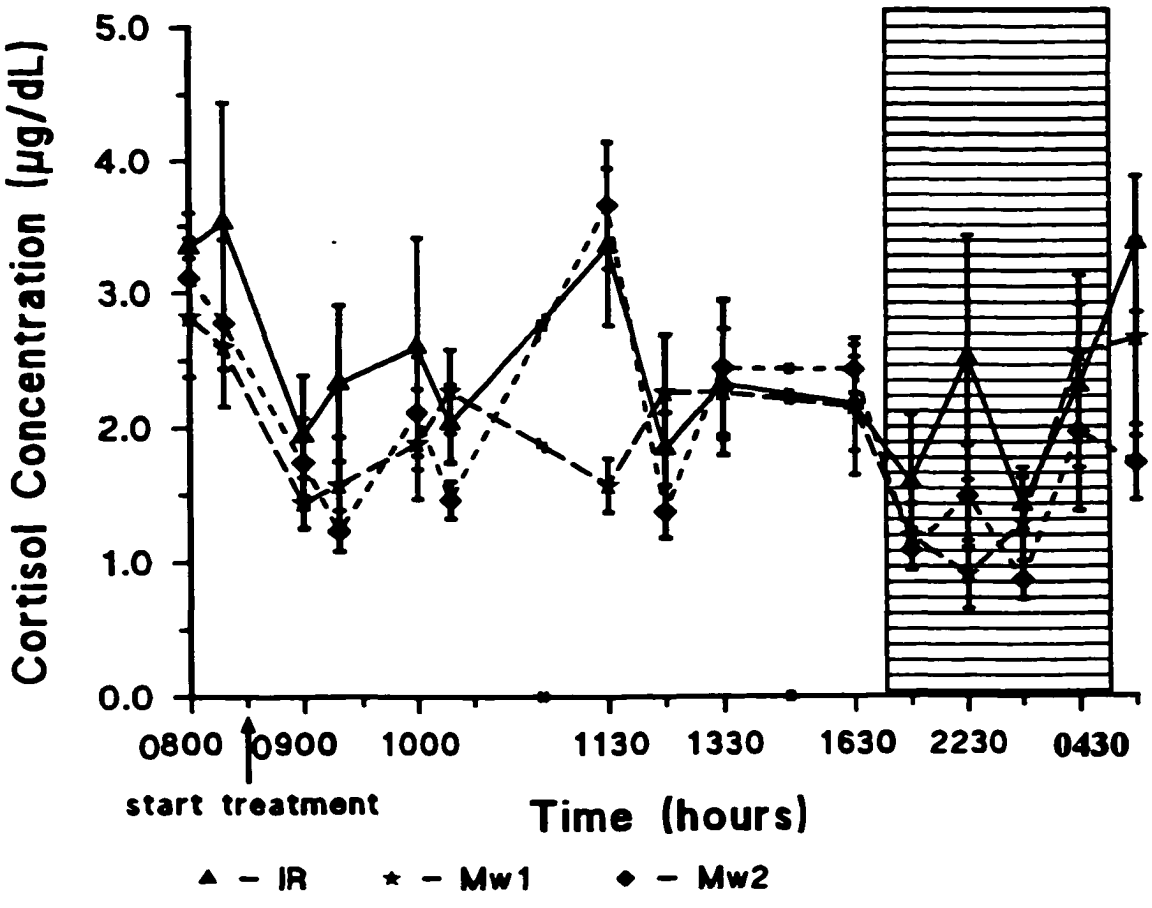


Figure 4. Mean Cortisol concentrations (µg dL^{-1}) of weaner pigs exposed to IR at 500 W (n = 11), Mw at 6.9 mW cm^{-2} (Mw1) (n = 6) and 3.8 mW cm^{-2} (Mw2) (n = 6). Shaded area represents the dark phase of the sampling period.

groups aside from these isolated peaks (range: 0.90 - 2.27μg dL^{-1}; 0.86 - 2.44μg dL^{-1}; and 1.41 - 2.60μg dL^{-1} for Mw1, Mw2 and IR groups, respectively and excluding the peak values). Furthermore, at each peak, plasma cortisol concentrations reached similar values both between peaks within treatment group and between treatment groups (3.35 ± 1.85μg dL^{-1} and 3.36 ± 1.52μg dL^{-1} for 1130 and 0730 h, respectively for IR; 3.66 ± 1.18μg dL^{-1} for 1130 h for Mw2; 2.66 ± 1.79μg dL^{-1} for 0730 for Mw1) (mean ± SD).

Significant differences ($P < 0.01$) were observed for pigs within treatment indicating significant individual variation (Table II). Significant differences were also noted between replicates which may also be a reflection of the individual variation or may be due to a consistent change in the environment or types of pigs used for each replicate.

3.4.3. Glucose data

Plasma glucose concentrations of Mw1-treated pigs differed ($P < 0.01$) from both Mw2- and IR-treated animals but the Mw2 group did not differ ($P > 0.05$) from the IR-treated group (Table IV). The mean glucose concentrations for Mw1-, Mw2-, and IR-exposed groups were 93.44 ± 0.87, 85.43 ± 0.87, and 82.76 ± 1.10 mg dL^{-1}, respectively. Significant ($P < 0.01$) pig within treatment and replicate effects were also noted (Table II). However, no differences ($P > 0.05$) in glucose concentrations were found between treatment groups prior to the onset of treatment (Table IV). Mean glucose concentrations at the start of blood sampling were 83.58 ± 6.01 mg dL^{-1}, 84.42 ± 6.01 mg dL^{-1} and 76.79 ± 4.92 mg dL^{-1} for Mw1, Mw2 and IR groups, respectively.

Table IV. *P* values for pretreatment and experimental analyses of variance testing differences in mean plasma glucose concentrations (mg dL^{-1}) in weaner pigs exposed to IR at 500 W (n = 11), Mw at 6.9 mW cm^{-2} (Mw1) (n = 6) and 3.8 mW cm^{-2} (Mw2) (n = 6).

	P values	
Treatments	Pretreatment	Experimental
Mw1[a] vs. IR[b]	0.3934	0.0001[d]
Mw2[c] vs. IR	0.3394	0.0570
Mw1 vs. Mw2	0.9230	0.0001[d]

[a] Microwave radiation at 6.9 mW cm^{-2}
[b] Infrared radiation at 250 W
[c] Microwave radiation at 3.8 mW cm^{-2}
[d] Significant at $P = 0.05$

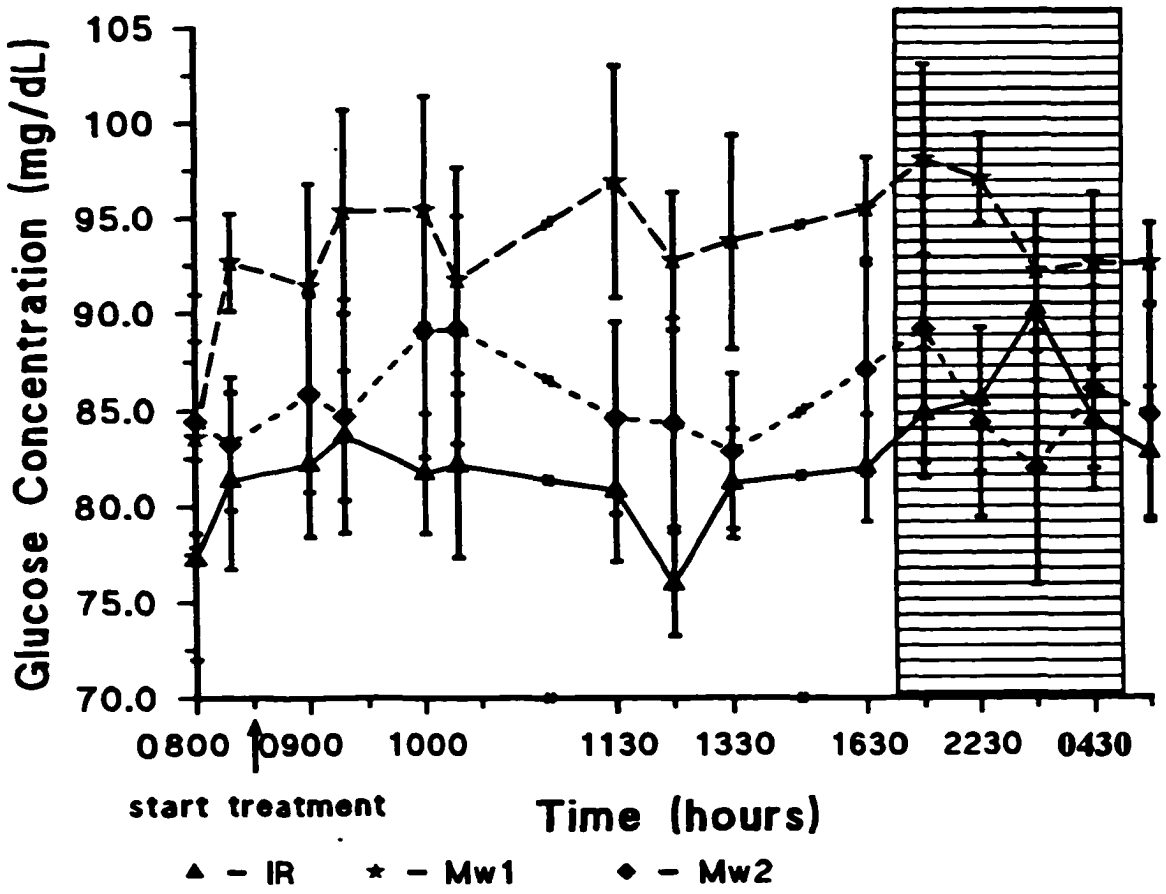


Figure 5. Glucose concentrations (mg dL^{-1}) in weaner pigs exposed to IR at 500 W (n = 11), Mw at 6.9 mW cm^{-2} (Mw1) (n = 6) and 3.8 mW cm^{-2} (Mw2) (n = 6).

Figure 5 depicts plasma glucose concentrations over a 24 hour period. In this graph it can be seen that plasma glucose concentration for all three treatment groups started out low and immediately increased at the start of the treatments, remaining at this higher level for the remainder of the sampling period (range: 91.42 - 98.08 mg dL^{-1}; 82.83 - 89.17 mg dL^{-1}; 76.35 - 90.13 mg dL^{-1} for Mw1, Mw2 and IR, respectively excluding the initial sample). This stability in glucose concentration over time is supported by the analysis of variance in which no significant time effect was found ($P > 0.05$). Furthermore, the lack of a time x treatment interaction (Table II) indicates that plasma glucose concentrations for all three treatment groups followed similar patterns over time. It is also apparent from Figure 5 that mean plasma glucose for the IR-treated animals remains consistently lower than the other two treatment groups for the entire 24 h period with the exception of two data points at 2230 h and 0100 h during which glucose values were higher than the Mw2 group. Glucose concentrations for the Mw1 treated group remains consistently higher than the other two treatment groups for the entire sampling period following the initial sample.

3.4.4. Correlations

No correlation was found between percent resting time and plasma melatonin concentration for pigs in replicate 1 ($P > 0.05$) (Table V). Furthermore, subjective observation of pig behaviour on the video tapes revealed no relationship between rising circulating melatonin and onset of sleep.

Table V. Pearson-correlation coefficient and *P* value for the comparison between plasma melatonin and percent rest time of weaner pigs exposed to IR at 500 W (n = 11), Mw at 6.9 mW cm^{-2} (Mw1) (n = 6) and 3.8 mw cm^{-2} (Mw2) (n = 6).

Variable	Sample size (n)	Correlation coefficient	*P* value
Plasma melatonin vs. % rest time	280	-0.1094	0.07

3.5 Discussion

In this study, plasma melatonin, cortisol and glucose concentrations of weaner pigs exposed to 915 MHz microwave radiation were measured to determine if microwave radiation alters these physiological parameters in a manner that is not observed in pigs exposed to nfrared radiation. The results of the ANOVA confirm that Mw alters circulating melatonin levels at average power levels of 6.9 mW cm^{-2} and 3.8 mW cm^{-2} when compared with melatonin levels of infrared-exposed weaner pigs. Microwave radiation at 6.9 mW cm^{-2} also significantly alters circulating glucose levels when compared to IR-exposed pigs. Plasma cortisol concentrations, however, were not altered by either Mw treatment when compared to cortisol values of IR-treated pigs.

The Mw-induced alteration in plasma melatonin was characterized by a higher mean melatonin concentration which appeared to be intensity-dependent. The highest mean circulating melatonin concentration was observed in pigs exposed to 6.9 mW cm^{-2} (16.26 $\pm$ 0.97 pg mL^{-1}) (mean $\pm$ SEM) followed by pigs exposed to 3.8 mW cm^{-2} (13.20 $\pm$ 1.05 pg mL^{-1}). The lowest mean circulating melatonin concentration was observed in the IR-exposed pigs (8.68 $\pm$ 0.85 pg mL^{-1}). However, mean melatonin concentrations measured before starting the treatments were not significantly different between treatment groups which indicates that the observed changes in melatonin levels was most likely due to a treatment effect and not due to individual variation. Furthermore, the treatment effect observed is most likely due to a physiological effect of Mw rather than an effect of light suppression of melatonin by IR. The following evidence supports a Mw effect: 1. a significant difference was detected between the Mw treatments which was intensity

dependent; 2. Melatonin concentrations for the Mw1-treated group remained higher than those of the Mw2-treated group during the daylight hours during which light intensity was higher in the Mw1-treated cage than in the Mw2-treated cage; 3. Prior to the onset of the treatment, melatonin concentrations in all three treatment groups were very similar and, at that time, light intensity in the Mw1 cage was higher than that in the Mw2 cage; 4. If the difference was due to light suppression of melatonin in IR-treated pigs, we would expect to see a decline in melatonin concentration at the onset of treatment which is not evident. However, melatonin concentrations for the Mw1-treated group increased following the onset of treatment; 5. According to Griffith and Minton, 1992, the light intensity used in this study (max. 106.7 lx) should not entrain pigs to the photoperiod and therefore should not affect plasma melatonin concentrations.

The finding of an increase in plasma melatonin concentration following exposure to electromagnetic radiation in this study is in contrast to the studies by Kato et al. (1993; 1994a) in which rats exposed to circularly polarized 50-Hz magnetic fields showed decreased concentrations of plasma and pineal melatonin. In addtion, a subsequent study by Kato et al. (1994b) revealed that there was no change in plasma or pineal melatonin concentrations following exposure to horizontal or vertical 50-Hz magnetic fields. The explanation proposed by Kato and associates in the latter paper that a " 'sweeping' or 'rotating' magnetic field stimulus with a complex, changing vector (is required to) interact with the geometry of the tissues of the pineal gland in a manner sufficient to produce an effect", can also be applied to the finding in this study. Since Mw is a combination of both electric and magnetic fields oriented at right angles to one another, this field configuration

may enable interaction with the pineal tissues in such a way as to increase production of melatonin. There are also other obvious differences between this study and those of Kato et al. (1993; 1994a; b) which may contribute to the different effects observed. First of all, the frequency of radiation used in this study was 915 MHz which is in the ultra-high frequency range and 18 million times greater than the 50 Hz used by Kato et al. (1993; 1994a; b). This higher frequency, or the shorter wavelength that results, may affect pineal tissues in a different manner than the 50 Hz used by Kato and associates. Secondly, as already stated, Mw has both an electric field and a magnetic field while the radiation used by Kato and associates (1993; 1994a; b) was described as magnetic. The electric field component of the Mw may be exerting a very different effect on the pineal gland either directly or indirectly through other structures such as the hypothalamus, the suprachiasmatic nucleus, the retina or the retino-hypothalamic tract. This effect may be the result of a structural change in brain or eye tissues or it may be the result of alterations at the subcellular level (i.e. biochemical effects, changes in organelles, altered receptor sites). Finally, another important difference between this study and those of Kato et al. (1993; 1994a; b) is the experimental subjects used. It has been demonstrated by several authors (Lynch, 1971; Wilkinson et al., 1977) that secretion of melatonin by the pineal gland in rats follows a circadian rhythm with increased secretion during the dark hours and decreased secretion during the daylight hours. However, inconsistent findings of melatonin secretion have been reported in pigs (Brandt et al., 1986; DeBoer and Hacker, 1987: McConnell and Ellendorff, 1987; Minton et al., 1989; Minton and Cash, 1990; Griffith and Minton, 1992; Bollinger et al., 1997). There may be a structural or functional difference between the

pineal glands of rats and pigs which may result in the differing patterns of melatonin secretion between these species in response to electromagnetic radiation exposure.

The plasma melatonin profile of Mw-exposed pigs demonstrated two peaks in circulating melatonin during the 24h period while IR-exposed pigs showed only one peak in circulating melatonin. The reason for this difference is difficult to explain at this time. One possible explanation for the double peak in the Mw1 group may be that the early peak which occurs during daylight hours is due to a direct or indirect effect of Mw on the pineal gland while the second peak which occurs during night time hours is the normal nocturnal peak corresponding to that seen in the IR-exposed pigs. One problem with this explanation is that the second peak occurs 6 hours later than the nocturnal peak seen in the IR group. However, this second peak could be delayed as a result of the elevation in melatonin production earlier in the day. The double peak demonstrated by the Mw2 group, however, does not follow a similar trend. In this situation the first peak occurs simulataneous with the peak seen in the IR group and they reach very similar concentrations. However, the second peak demonstrated by the Mw2 group occurs during the early morning hours after the lights are back on. This peak may be falsely elevated as a result of high melatonin levels measured in one pig in replicate 1. This explanation is supported by the fact that this peak is not evident in the melatonin profile of Mw2-exposed pigs in replicate 2 (Figure 3). Failure of the Mw2 group to show an early peak during daylight hours may be explained by the lower Mw power level used in this treatment which may have been insufficient to induce such a dramatic alteration in melatonin excretion by the pineal gland.

As previously mentioned, a daily pattern of increased circulating melatonin during afternoon and evening hours and decreased circulating melatonin during morning hours was demonstrated by pigs in this study. However, not all of the animals demonstrated a nocturnal rise in plasma melatonin. This is similar to the study by Bollinger et al. (1997) in which only 16% of gilts kept on a 12h:12h light dark cycle displayed a nocturnal rise in serum melatonin. Furthermore, of those that did show a nocturnal rise in circulating melatonin in the present study, some showed a large increase in melatonin concentrations from day time values while others showed only a slight increase in melatonin concentrations during the night time hours. Similarly, McConnell and Ellendorff (1987) reported a nocturnal increase in serum melatonin in 3 sows for which the peak values obtained varied significantly (190, 294 and 546 pg mL^{-1}) and for which the magnitude of the elevation ranged from 2- to 5-fold over peak daytime values. Obviously, the peak mean melatonin concentration obtained in this study (26.28 pg ml^{-1}) is much lower than those reported by McConnell and Ellendorf (1987). This may be explained, in part, by the age difference of pigs used in these two studies (weaners vs sows). The variation may also be due to differences in the assays used to measure plasma melatonin. The RIA used by McConnell and Ellendorf (1987) was validated for use with pig plasma while the RIA used in this study was based on human plasma samples. Therefore, our results may be less accurate than those obtained by McConnell and Ellendorf (1987). However, the differences may also be due to simple individual variation. This is supported by the finding by McConnell and Ellendorf (1987) of peak melatonin concentration during the dark phase of a 12:12 h light:dark cycleof < 16 - 122 pg ml^{-1} in a second experiment in the same paper. Based on the findings

of this study and those of other researchers (McConnell and Ellendorff, 1987; Bollinger et al. 1997), it is obvious that the circadian rhythmicity in melatonin secretion seen in most other mammals is not a consistent finding in swine. This inconsistency explains the individual variation seen in this study and accounts for the significant pig within treatment effect detected in the ANOVA. Another possible contributing factor to the wide variation in melatonin concentrations between pigs in this study is analytical variability. Large intra- and interassay variation was present as demonstrated by the coefficients of variation (C.V.) (17.3%, 30.8% and 20.6% for intraassay C.V. and 28.3% for interassay C.V.). Furthermore, the assay standards used to create the standard curve were human serum samples. Since the accuracy of this assay has not been demonstrated using pig serum samples, some of this large variability may be accounted for by the limitations of the assay with respect to pig blood.

The analysis of circulating melatonin concentration was further complicated by the large number of assay results (57 single samples and 36 duplicate samples) which were off the standard curve and therefore considered unreliable. Because these values were unreliable they could not be used in the statistical analysis. However, in order to maintain an adequate sample size per blood sampling time, the corresponding 57 single samples were used in the analysis. Obviously, this may be inappropriate but was justified by making use of the half life of melatonin in blood and the preceding and subsequent melatonin values as guidelines to determine the potential accuracy of these samples. Given the large individual variation in melatonin concentration between pigs, it was deemed necessary to use as many samples as possible to detect a Mw effect on melatonin concentration.

It has been demonstrated by Cagnacci et al. (1992) that in humans, daily peak body core temperature is followed by a decline in body core temperature that is inversely correlated with the rise in serum melatonin levels suggesting that melatonin may be an important regulator of body core temperature. This is one possible mechanism by which Mw is affecting circulating melatonin levels. However, if core body temperature were elevated in these pigs, they should be showing signs of heat stress, such as laying with max. contact with the floor, panting, drinking excessively, or running water over themselves with the drinkers. None of these behaviours were observed in the pigs used in this study. On the other hand, it is possible that inactivity was sufficient to prevent heat stress in the face of slightly elevated core body temperature. Unfortunately, body core temperatures were available for an inadequate numbers of pigs and therefore, no conclusions can be drawn regarding body temperature elevations.

It is also conceivable that the Mw may be warming the hypothalamus directly resulting in increased melatonin secretion. The relationship between warming of the hypothalamus and prolonged sleep time in kangaroo rats has been reported by Sakaguchi et al. (1979). In addition, Preoptic-anterior hypothalamic (POAH) lesion-induced insomnia in cats was shown to be reversed by exposure to heat and improved sleep was associated with elevated brain temperatures (Szymusiak et al., 1991). Furthermore, the relationship between melatonin secretion and sleep onset has been demonstrated by Dollins et al. (1994) and others. In the study by Dollins et al.(1994), oral temperature decreased following administration of melatonin. Circulating melatonin was also shown to be correlated with a maximal decline of core body temperature at night by Cagnacci et al. (1992). Given the

relationships between brain temperature and sleep, between sleep and melatonin secretion and between melatonin secretion and body core temperature, it is possible that warming of the hypothalamus may result in melatonin secretion causing somnolence and a subsequent drop in hypothalamic and body core temperature. This may also explain why body core temperature and circulating plasma melatonin concentration show similar trends in the pigs implanted with the radiotransmitter (data not shown).

The absence of a microwave effect on plasma cortisol levels suggests that microwave radiation at 6.9 mW cm^{-2} and 3.8 mW cm^{-2} does not act as a stressor in 4 and 5 week old, recently weaned pigs. This is comparable to the findings of Lotz and Michaelson (1978), in which 2 hours of microwave exposure at 13 mW cm^{-2} (2450 MHz, CW) did not result in elevated corticosterone levels in rats. However, elevations in corticosterone levels were noted in the same study at higher Mw power levels and these elevations were highly correlated with increased colonic temperature. In 1973, Parker also reported no change in circulating corticosterone levels in rats exposed to 10 mW cm^{-2} microwave radiation. On the other hand, several investigators have noted increased corticosteroid levels following microwave radiation. For example, Petrov and Syngayevskaya (1970) found that corticosteroid levels in dog blood increased by 100 and 150% after 3 and 24 h exposures, respectively, to 3000 MHz at 10 mW cm^{-2} microwaves. Likewise, Guillet et al. (1975) demonstrated that plasma corticosterone increases within 15-30 minutes of the start of Mw exposure and falls sharply within 15-30 minutes after termination of exposure. Further evidence against the concept of Mw acting as a stressor in 4 and 5 week old pigs is the finding that, following the onset of Mw, plasma cortisol

concentrations for both Mw-treated groups decreased. This may reflect improved thermal conditions for the pigs following a period of exposure to a cold environment. Reduced environmental temperature was necessary in order to prevent heat exhaustion upon exposure to microwave radiation. The drop in cortisol concentration was noted immediately in Mw-exposed animals, whereas in IR-exposed animals cortisol levels initially increased at the start of the treatment. This may reflect the delay in return to thermal comfort by pigs in the IR treatment due to the time required to transfer heat from the body surface to the body core. Since Mw's penetrate deeper into the animal's body, thermal comfort would be attained much faster in the Mw-treated group.

The cortisol profiles for each group of pigs fail to demonstrate a pattern that is consistent with the normal circadian rhythm of cortisol in pigs. It has been demonstrated by numerous investigators that cortisol concentrations in pigs show a circadian rhythmicity in which concentrations are higher in the morning hours and lower in the afternoon hours (Whipp et al., 1970; Rafai and Fodor, 1980; Barnett et al., 1981; and Becker et al., 1985). This finding is not surprising, however, since this circadian rhythm develops with age and, at 4 weeks of age, pigs show no sign of diurnal variation in cortisol profiles (Evans et al., 1988). In fact, adult plasma cortisol profiles are not established in gilts until approximately 24 weeks of age (Evans et al., 1988). In the absence of established circadian rhythmicity in circulating cortisol levels it is impossible to say whether microwave radiation has had any effect on the rhythm of cortisol secretion in the pigs in this study.

The mean concentrations of cortisol observed in all three treatment groups (2.4, 1.96 and 1.96 $\mu g\ dL^{-1}$ for IR, Mw1 and Mw2, respectively) are below the reported resting

cortisol concentration of 2.53 $\mu g\ dL^{-1}$ for 4 week old piglets (Evans et al., 1988). This suggests that the pigs used in this study were perhaps no longer under any stress associated with the surgery or being individually housed.

Plasma glucose concentration is often used as an indicator of stress. This elevation in circulating glucose is due, in part, to the gluconeogenic effect of glucocorticoids but also to the interference of insulin's activity on cells by cortisol, resulting in decreased glucose uptake by cells. In this study, mean plasma glucose concentration of pigs exposed to 6.9 $mW\ cm^{-2}$ (Mw1) was higher than that of both IR and Mw2-exposed pigs. However, this is not likely associated with a stress response since the cortisol concentrations were not elevated. One possible explanation for the elevated glucose concentration in Mw-exposed pigs is that the microwaves may have a stimulatory effect on growth hormone (GH) secretion resulting in inhibition of glucose uptake by cells. Although GH was not measured in this study, this hormone has been shown to increase in the circulation of rats following exposure to 2.45 GHz microwaves at 13 $mW\ cm^{-2}$ (Michaelson et al., 1975).

3.6 CONCLUSION

Microwave radiation at 3.8 $mW\ cm^{-2}$ and 6.9 $mW\ cm^{-2}$ will increase circulating levels of melatonin in an intensity-dependent fashion in weaner pigs. However, this alteration in plasma melatonin concentration does not appear to be associated with thermal stress or other types of stress as indicated by plasma cortisol levels comparable to IR-exposed pigs. Microwave exposure at 6.9 $mW\ cm^{-2}$ is associated with elevated plasma

glucose concentrations, however, in the absence of elevated cortisol levels. In order to determine the cause of this elevation in glucose, further work should be done using similar power levels of microwave exposure.

4.0 GENERAL DISCUSSION

In the experiment discussed in chapter 2, the reduced activity level observed in weaner pigs exposed to microwave radiation did not appear to have an adverse effect on the animals. Microwave-exposed pigs appeared to be resting comfortably with no signs of chilling, heat stress or other forms of stress due to the microwave radiation. Although, pigs were apparently comfortable with respect to the effective environmental temperature, it is possible that the reduced activity level observed in Mw-exposed pigs was a form of behavioural thermoregulation which was sufficient to prevent heat stress. However, the reduction in activity level was power level dependent and, therefore, could likely be fine-tuned until a power level is reached which is optimum for maintenance of thermal comfort and allows maximum growth rates in weaner pigs. Although the growth rates observed in this study were statistically comparable between microwave- and infrared-exposed pigs, slightly lower growth rates were observed in microwave-exposed pigs which were less obvious at the lower microwave power level. This suggests that the difference in weight gain may not have been just due to chance and may be economically significant to the producer. It is possible that the inactivity displayed by the Mw-exposed pigs also meant less time spent eating and therefore, less food consumed. This could explain the lower ADG of the Mw-exposed pigs and suggests a negative effect of Mw on activity level. However, there may be an optimum Mw power level at which activity level would be reduced only enough to reduce food energy requirements without suppressing growth. If this is true a Mw-induced suppression of activity could be advantageous and more desirable to producers.

In the second experiment, microwave-exposed pigs had increased average circulating melatonin concentrations compared to infrared-exposed pigs. The objective of measuring melatonin levels was to determine if the reduced activity level observed in the first experiment was mediated through pathways which resulted in increased production of the sleep-inducing hormone, melatonin. One important distinction that should be made here is that, in the first experiment, activity level was measured as percent resting time which is not necessarily sleeping time. Measurement of sleeping time was not possible since behaviour was assessed from videotaped recordings and it was not always possible to determine if the animals were sleeping. In the second experiment, the assumption was made that melatonin would increase resting time and thereby reduce activity level as a result of its sleep-inducing effect. It should be noted, however, that the power levels of Mw used in the second experiment were different from the first experiment. As already explained, the power levels recorded were the averages over the experimental period. One of the power levels used (128.6 W) was comparable to the low Mw power level used in the first experiment (109 W) but the second power level had no comparison in the first experiment. Although an alteration in circulating melatonin levels was noted at this lower power level (70.7 W), it is difficult to correlate this with a reduction in activity level since the pigs in the second study were only exposed to Mw over a 24 h period. However, the finding of increased melatonin in these pigs warrants further investigation to determine the effect of Mw on activity level and growth at a similar power level.

Although microwave-exposed pigs had higher average circulating melatonin levels that appeared to be intensity-dependent, there was no obvious immediate relationship

between melatonin levels and pig resting behaviour. Videotaped recordings of pig behaviour at times when peak melatonin levels were measured often revealed active behaviour while low melatonin concentrations were sometimes accompanied by resting behaviour. In other species peak melatonin concentrations occur during sleeping hours and troughs in circulating melatonin levels occur during waking hours (Lynch, 1971; Reiter, 1986). It is possible that the effect of melatonin in pigs is cumulative resulting in a delay in the onset of sleep in this species. It is known that pigs do not consistently display a circadian rhythm of melatonin secretion as is seen in other species in which melatonin concentrations are highest at night and lowest during daylight hours (Brandt et al., 1986; McConnell and Ellendorff, 1987). A cumulative effect of melatonin action at a specific target site in the brain may explain this species variation in melatonin rhythm. Furthermore, this may explain the lack of a correlation between percent resting time and melatonin concentration over a 24 hour period. It would be interesting to see if any correlation existed between resting time and melatonin levels over a longer time period.

Although we were not able to demonstrate a relationship between activity level in weaner pigs and circulating melatonin levels, this study does provide evidence that exposure to microwave radiation results in higher circulating melatonin concentrations in a intensity-dependent manner. The mechanism by which microwave radiation induces this effect is unknown. However, several possible explanations can be speculated. It is possible that the microwave radiation is directly stimulating one or more of the areas of the brain that are involved in melatonin production (i.e. the pineal gland, the superior cervical ganglion, the spinal cord, the medial forebrain bundle, the hypothalamus or the SCN). This is a

possibility since microwaves have good penetration through bone and there is very little muscle mass or fat over the skull. Another possible mechanism of action of microwaves is via the retina. Stimulation of the retina may result in impulses travelling along the retinohypothalamic tract and blocking inhibition of NAT and HIOMT activity. This would allow melatonin production to occur without restriction during daylight hours. Another possible explanation involving the retina is that the microwave radiation may be interfering with light stimulation of the retina thereby preventing light suppression of melatonin production. These explanations are based on the assumption that melatonin production in the pig is under similar control mechanisms as in other species regardless of the fact that the circadian rhythm is not consistently present in this species. As already mentioned, these proposed mechanisms of action are purely speculative as insufficient information has been obtained in these experiments to draw any definite conclusions. Likewise, we are not able to draw conclusions regarding the Mw-induced effect on activity level in weaner pigs based on the results obtained in these experiments. However, we can not rule out the possibility that the reduction in activity level may be due to stimulation of melatonin production by microwaves. Perhaps a follow-up study in which activity level, circulating melatonin concentration, and NAT and HIOMT activity are quantified over a one week duration would provide a better understanding of the role of melatonin in activity level of weaner pigs. Such an experiment may also provide an understanding of the mechanism by which the pigs adapt to the Mw with respect to activity level.

Another possible mechanism by which Mw causes reduced activity in weaner pigs is through direct heating of the hypothalamus. Microwave radiation has been shown to have

a heating effect on tissue through the production of friction by vibrating polar molecules (Copson, 1975). Direct heating of the hypothalamus in kangaroo rats increased total sleep time while cooling of the hypothalamus at the same ambient temperature resulted in decreased total sleep time (Sakaguchi et al., 1979). In a subsequent study by Szymusiak et al. (1991), insomnia induced by POAH lesions was reversed by exposure to heat. Furthermore, it has been shown by Boulant and Dean (1986) that the predominant thermosensitive cell type in the POAH are warm-sensitive neurons. The findings of Szymusiak and collegues (1991) suggest that the facilitatory effect of elevated brain temperature on sleep is mediated by the POAH. The role of sleep in thermoregulation has also been reported by several investigators (Aschoff and Wever, 1981; Barret et al., 1987; Wever, 1989) who have demonstrated a decline in body core temperature following sleep onset. Based on the relationship between hypothalamic heating, sleep onset and body core temperature, it seems reasonable to hypothesize that direct microwave heating of the hypothalamus may be inducing sleep onset as a form of thermoregulation thus resulting in the reduced activity noted in Mw-exposed pigs.

The thermoregulatory hypothesis of the reduced activity level can also explain the elevated circulating melatonin levels observed in Mw-exposed pigs in the second experiment. In 1992, Cagnacci and associates demonstrated that melatonin is a major regulator of the circadian rhythm of body core temperature in humans. In that study, the circadian decline of body core temperature was shown to be coupled with serum melatonin levels independent of the sleep-wake cycle. Furthermore, they hypothesized that the close temporal association between sleep and melatonin may result in an additive hypothermic

effect. In this study, elevated hypothalamic temperature may have initiated a dual effect of sleep onset and increased melatonin production in an attempt to rapidly return hypothalamic temperature to its set point temperature (Hammel et al., 1963). Furthermore, prolonged exposure to Mw may have resulted in an alteration in the set point temperature at which thermoregulatory mechanisms are activated. This would explain the adaptation to Mw observed in the first experiment in which the activity level of Mw-exposed pigs gradually returned to pretreatment levels. In this case we would expect to see an elevation in body core temperature as thermoregulatory mechanisms shut down.

An alternative, or perhaps coincident, mechanism by which pigs adapt to the relaxing effect of Mw may be through the down regulation of melatonin receptors. Down regulation of melatonin receptors in pars tuberalis cells of the anterior pituitary in sheep following prolonged exposure (24 h) to melatonin has been demonstrated by Hazlerigg et al. (1993).

The proposed theory that the Mw-induced reduction in activity is a thermoregulatory mechanism might suggest that the animals were experiencing heat stress. However, there was no evidence that the animals were experiencing discomfort. Pigs in the first experiment sometimes spread themselves out around the cage in recumbent postures but this behaviour has also been noted in pigs kept within a thermoneutral environment (Mount, 1968). Furthermore, Mw-exposed pigs showed no signs of increased respiration or panting which suggests that they were having no difficulty maintaining thermal comfort. In rats, Mw exposure that resulted in elevated colonic temperature was associated with increased locomotor activity, restlessness and eventual escape behaviour at high power levels (Lotz

and Michaelson, 1978). This type of behaviour was not evident among the pigs used in this study. Further evidence that Mw-exposed pigs were not experiencing thermal stress comes from the finding that serum cortisol levels in these animals were comparable to those of IR-exposed animals in the second experiment. In fact, although the difference was not statistically significant, plasma cortisol levels of Mw exposed pigs were lower than those of IR exposed pigs suggesting that Mw exposure is even less stressful than IR exposure at the power level used in this study.

Although there was no evidence of stimulation of the hypothalamo-pituitary-adrenal axis by Mw in this study, Mw-exposed pigs did show elevated plasma glucose levels at the high power level (6.9 mW cm^{-2}) in the second experiment. This elevation in glucose is not due to the gluconeogenic effect of cortisol since cortisol values were not elevated. There are several possible explanations for this elevation in plasma glucose. The first explanation is based on the assumption that the reduced activity level is due to a somnogenic effect of Mw, either via melatonin production or as a thermoregulatory mechanism. It is well known that GH is released in phasic bursts shortly after sleep onset (Martin, 1973; Schally et al., 1973). One of the actions of GH is that it antagonizes the effects of insulin causing inhibition of cellular uptake of glucose and release of free fatty acids from tissue storage depots (Michaelson and Lin, 1987; Walton and Etherton, 1986). If Mw does, in fact, have a somnogenic effect on pigs, the elevated glucose may be the result of subsequent GH release. On the other hand, release of GH by the pituitary is regulated by a releasing factor (Growth hormone releasing factor) (GRF) and an inhibiting factor (somatostatin) which originate in the hypothalamus (Schally et al., 1973). The area of the hypothalamus from

which these factors originate is intimately associated with the hypothalamic region that is involved in temperature regulation (Martin, 1973; Schally et al., 1973). If Mw energy is directly stimulating the hypothalamus effecting a thermoregulatory response, it may also be stimulating GRF resulting in elevated circulating GH and subsequent elevated glucose concentrations. In fact, it is conceivable that these two mechanisms may be occurring simultaneously. It would be interesting to measure circulating GH levels in a similar follow-up study. In rats exposed to 2450 MHz Mw at 13 mW cm^{-2}, plasma GH levels increased but at 36 mW cm^{-2}, GH levels drop after 60 min of exposure (Michaelson et al., 1975). This reversal in GH secretion at higher power densities may explain the lower growth rates of Mw exposed pigs in experiment 1. The power densities used in that experiment were 11.4 mW cm^{-2} and 6.1 mW cm^{-2} for Mw1 and Mw2, respectively. In experiment 2, the power densities used were 6.9 mW cm^{-2} and 3.8 mW cm^{-2} for Mw1 and Mw2, respectively. If Mw is proven to have a stimulatory effect on GH secretion, this may be an added beneficial effect of this technology in that it may increase growth rates of weaner pigs.

In summary, 915 MHz Mw at power densities up to 11.4 mW cm^{-2} does not appear to have any detrimental effects on newly weaned pigs. Further studies are required, however, to ensure that detrimental effects have not been overlooked in these experiments, such as effects on brain tissue, the retina and long term effects on growth rates. In addition, it would be beneficial to investigate the effect of Mw upon GH secretion and its effect on average daily gain in weaner pigs. Finally, subsequent studies should be conducted to determine the optimal Mw power density that would maximize benefits and minimize adverse effects.

5.0 APPENDIX A

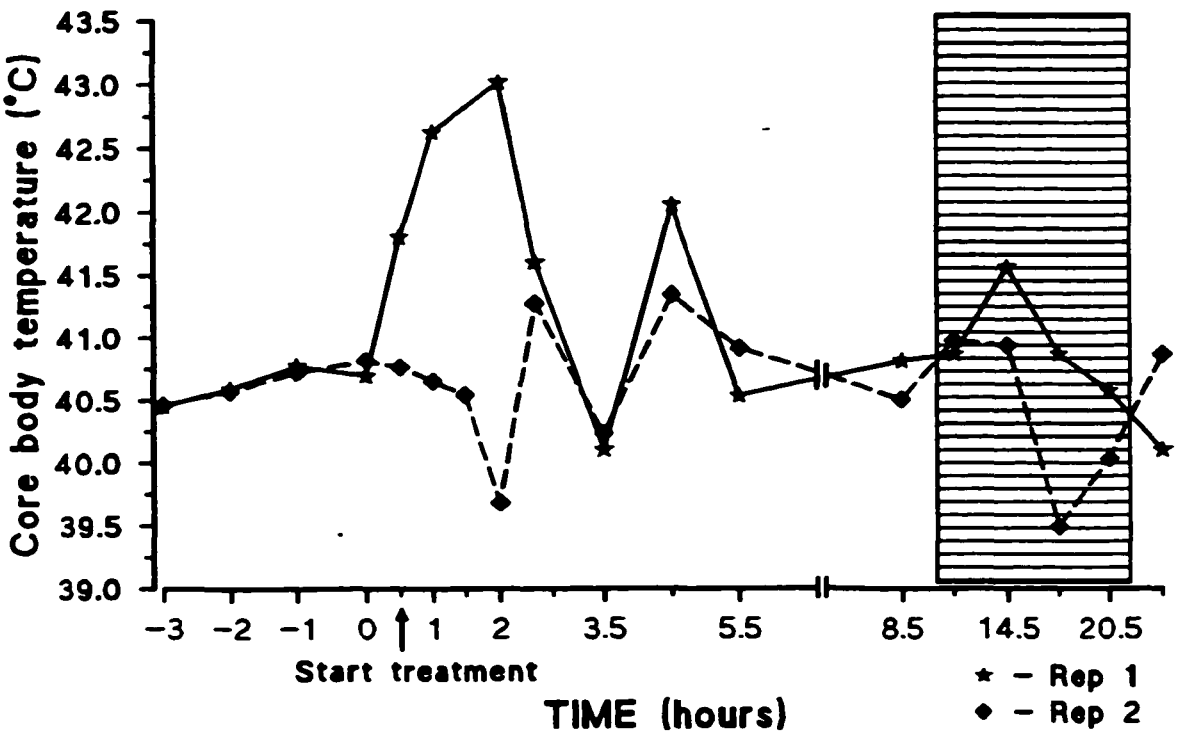


Figure 1. Body core temperatures (°C) during the pretreatment (time = -3,-2 and -1 days before treatment) and treatment (time = 0.5 - 23.5 hrs) periods for replicates 1 (Mw2) (3.8 mW cm^{-2}) (n=1) and 2 (Mw2) (n=1). Time 0 = time of first blood sample.

The graph of body core temperatures over time (Figure 1) show that during the pretreatment period body core temperatures were increasing only slightly and were very similar in both replicates. However, after the onset of treatment, body core temperature of the pig in replicate 1 showed a drastic increase while that of the pig in replicate 2 showed a very slight decrease. By 3.5 h into the treatment body core temperatures of both animals were again very similar and at a lower temperature than they had been in the pretreatment period. After this sampling time both pigs showed mild fluctuations in body core temperature which followed similar patterns until the second last recording. At this time body core temperature of the pig in replicate 2 was increasing and that of the pig in replicate 1 was decreasing.

6.0 REFERENCES

Acorn. R.K. 1996. Effects of microwave energy as a source of supplemental heat on growth, behaviour, and reproduction of weaner pigs . MSc Thesis, University of Guelph.

Adair, E.R. 1983. Sensations, Subtleties, and Standards: Synopsis of a panel discussion. In: Adair, E.R., ed. Microwaves and Thermoregulation. Academic Press, New York, NY. Pp.231-238.

Ader, R. 1962. Social factors affecting emotionality and resistance to disease in animals: III. Early weaning and susceptibility to gastric ulcers in the rat, a control for nutritional factors. J. Comp. Physiol. Psychol. 55:600-606.

Adey, W. R. 1980. Frequency and power windowing in tissue interaction with weak electromagnetic fields. Proc. IEEE 60: 119-125.

Agriculture and Agri-Food Canada Publication 1898/E. 1993. Recommended code of practice for the care and handling of farm animals: Pigs. Minister of Supply and Services Canada, Ottawa, Ont.

Alexander, T.J.L., Thornton, K., Boon, G., Lysons, R.J. and Gush, A.F. 1980. Medicated early weaning to obtain pigs free from pathogens endemic in the herd of origin. Vet. Rec. 106:114-119.

Analytical Software. 1992. Statistix. Version 4.0.

ANSI. 1988. American National Standards Institute. American national standard safety levels with respect to human exposure to radiofrequency electromagnetic fields, 3 kHz to 300 GHz. Draft No. 3, 1988 Revision, June 1988. New York, Institute of Electrical and Electronic Engineers, Inc.

Anton-Tay, F., Diaz, L. and Fernandez-Guardiola, A. 1971. On the effect of melatonin upon human brain: its possible therapeutic implications. Life Sci. 10:841-850.

Aschoff, J. and Wever, R.1981. The circadian system of man. In: Aschoff, J., ed. Handbook of Behavioral Neurobiology and Biological Rhythms. Plenum Press, New York, NY. Pp.311-331.

Axelrod, J. and Weissbach, H. 1960. Enzymatic O-methylation of N-acetylserotonin to melatonin. Science 131:1312-1313.

Baldwin, B.A. and Ingram, D.L. 1967. The effect of heating and cooling the hypothalamus on behavioural thermoregulation in the pig. J. Physiol. (London) 191:375-392.

Barber, R.S., Braude, R. and Mitchell, K.G. 1955. The value of electrically warmed floors for fattening pigs. J. Agric. Sci., Camb. 46:31-36.

Barchas, J., DaCosta, F. and Spector, S. 1967. Acute pharmacology of melatonin. Nature 214:919-920.

Barnett, J.L., Winfield, C.G., Cronin, G.M. and Makin, A.W. 1981. Effects of photoperiod and feeding on plasma corticosteroid concentrations and maximum corticosteroid binding capacity in pigs. Aust. J. Biol. Sci. 34:577-585.

Barret, J., Morris, M. and Lack, L. 1987. The sleep evoked decrease of body temperature. Sleep Res. 16:569 (Abstract).

Bate, L.A., Crossley, J.G. and Wade, R. 1992. Effective rewarming of hypothermic piglets using 915 MHz microwaves. Can. J. Anim. Sci. 72:161-164.

Batini, C., Moruzzi, G., Palestini, M., Rossi, G.F. and Zanchetti, A. 1958. Persistent patterns of wakefulness in the pretrigeminal midpontine preparation. Science 128:30-32.

Batini, C., Magni, F., Palestini, M., Rossi, G.F. and Zanchetti, A. 1959a. Neural mechanisms underlying the enduring EEG and behavioural activation in the mid-pontine pretrigeminal cat. Arch. Ital. Biol. 97:13-25.

Batini, C., Moruzzi, G., Palestini, M., Rossi, G.F., and Zanchetti, A. 1959b. Effects of complete pontine transections on the sleep-wakefulness rhythm: the midpontine pretrigeminal preparation. Arch. Ital. Biol. 97:1-12.

Becker, B.A., Knight, C.D., Buonomo, F.C., Jesse, G.W., Hedrick, H.B. and Baile, C.A. 1992. Effect of a hot environment on performance, carcass characteristics, and blood hormones and metabolites of pigs treated with porcine somatotropin. J. Anim. Sci. 70:2732-2740.

Becker, B.A., Minaber, J.A., Christenson, R.K., Manak, R.C., DeShazer, J.A. and Hahn, G.L. 1985. Peripheral concentrations of cortisol as an indicator of stress in the pig. Am. J. Vet. Res. 46:1034-1038.

Beilharz, R.G. and Cox, D.F. 1967. Social dominance in swine. Anim. Behav. 15:117-122.

Berruecos, J.M., Dillard, E.U. and Robison, O.W. 1970. Selection for low backfat thickness in swine. J. Anim. Sci. 30:844-848.

Binkley, S., Klein, D.C. and Weller, J. 1974. Dark induced increase in pineal serotonin N-acetyltransferase activity: a refractory period. Experientia (Basel) 29:1339-1340.

Blackwell, R.P. and Saunders, R.D. 1986. The effects of low-level radiofrequency and microwave radiation on brain tissue and animal behaviour. Int. J. Radiat. Biol. 50:761-787.

Blecha, F., Pollmann, D.S. and Nichols, D.A. 1983. Weaning pigs at an early age decreases cellular immunity. J. Anim. Sci. 56:396-400.

Bojkowski, C.J. 1988. 6-Sulphatoxymelatonin as an index of pineal function in human physiology. Thesis, University of Surrey, UK.

Bollinger, A.L., Wilson, M.E., Pusateri, A.E., Green, M.C., Martin, T.G. and Diekman, M.A. 1997. Lack of a nocturnal rise in serum concentrations of melatonin as gilts attain puberty. J. Anim. Sci. 75:1885-1892.

Boulant, J. A. 1981. Hypothalamic mechanisms in thermoregulation. Fed. Proc. 40:2843-2850.

Boulant, J.A. and Dean, J.B. 1986. Temperature receptors in the central nervous system. Annu. Rev. Physiol. 48:639-654.

Braithwaite, L.A., Morrison, W.D., Otten, L. and Bate, L.A. 1994. Microwave radiation as a heat source for young livestock. In: Collins, E. and Boon, C., eds. Livestock Environment IV. Pp. 609-616.

Braithwaite, L.A., Morrison, W.D., Widowski, T., Duncan, I.J.H., Otten, L. and Bate, L.A. 1992. Behavioural observations of weaner pigs exposed to microwave radiation. Can. J. Anim. Sci. 72:1007 (Abstract).

Brandt, K. E., Diekman, M. A., Green, M. L. and Malayer, J. R. 1986. Lack of a circadian rhythm in serum concentrations of melatonin in prepubertal gilts. J. Anim. Sci. 63(suppl. 1):356 (Abstract)

Bremer, F. 1935. Cerveau "isolé" et physiologie du sommeil. C.R. Soc. Biol. 118:1235-1241.

Bremer, F. 1937. L'activité cerebral au cours du sommeil et de la narcose. Contribution à l'étude du mecanisme du sommeil. Bull. Acad. R. Med. Belg. 4:68-86.

Bremer, F. 1938. L'activité électrique de l'écorce cerebral et le problème physiologique du sommeil. Boll. Soc. Ital. Biol. Sper. 13:271-290.

Brown, A.C. and Brengelmann, G.L. 1970. The interaction of peripheral and central inputs in the temperature regulation system. In: J.D. Hardy, A.P. Gagge, and J.A.J. Stolwijk, eds. Physiological and behavioral temperature regulation. Charles C. Thomas, Springfield, Ill. Pp. 684-702.

Brown, R., Young, S. N., Gauthier, S., Tsuih, H. and Grota, L. J. 1979. Melatonin in human cerebrospinal fluid in daytime: its origin and variation with age. Life Sci. 25:929-936.

Burlacu, G., Băia, G., Ionilă, D., Moisa, D., Taşcenco, V., Vişan, I. and Stoica, I. 1973. Efficiency of the utilization of the energy of food in piglets, after weaning. J. Agric. Sci., Camb. 81:295-302.

Cagnacci, A., Elliott, J.A. and Yen, S.S.C. 1992. Melatonin: A major regulator of the circadian rhythm of core temperature in humans. J. Clin. Endocrinol. Metab. 75:447-452.

Cagnacci, A., Soldani, R. and Yen, S. S. C. 1993. The effect of light on core body temperature is mediated by melatonin in women. J. Clin. Endocrinol. Metab. 76:1036-1038.

Cameron, N.D. and Curran, M.K. 1994. Selection for components of efficient lean growth rate in pigs 4. Genetic and phenotypic parameter estimates and correlated rsponses in performance test traits with *ad libitum* feeding. Anim. Prod. 59:281-291.

Carroll, D.R., Levinson, D.M. and Justesen, D.R. 1980. Failure of rats to escape from a potentially lethal MW field. Bioelectromagnetics 1:101-115.

Cespuglio, R., Faradji, H., Riou, F., Buda, M., Gonon, F., Pujol, J.F. and Jouvet, M. 1981. Differential pulse voltammetry in brain tissue. II. Detection of 5-hydroxy-indoleacetic acid in the rat striatum. Brain Res. 223:299-311.

Chou, C.K. and Guy, A.W. 1979. Microwave-induced auditory responses in guinea pigs: relationship of threshold and microwave-pulse duration. Radio Sci. 14(suppl.6):193-197.

Chou, C.K., Yee, K.C. and Guy, A.W. 1985. Auditory response in rats exposed to 2450 MHz electromagnetic fields in a circularly polarized waveguide. Bioelectromagnetics 6:323-326.

Christon, R. 1988. The effect of tropical ambient temperature on growth and metabolism in pigs. J. Anim. Sci. 66:3112-3123.

Clark, L.K. and Leman, A.D. 1987. Factors that influence litter size in swine: Parity 3 through 7 females. J. Am. Vet. Med. Assoc. 191:187-194.

Cleveland, E.R., Cunningham, P.J. and Peo, E.R. 1982. Selection for lean growth in swine. J. Anim. Sci. 54:719-727.

Coindet, J., Chouvet, G. and Mouret, J. 1975. Effects of lesions of the SCN on paradoxical sleep and slow wave sleep circadian rhythms in the rat. Neurosci. Lett. 1:243-247.

Coetzee, J.A., Theron, J.J. and Van der Merwe, C.A. 1989. Consecutive melatonin circadian rhythms in normal volunteers. S. Afr. Med. J. 75:163-165.

Cole, D. J. A., Varley, M. A. and Hughes, P. E. 1975. Studies in sow reproduction 2. The effect of lactation length on the subsequent reproductive performance of the sow. Anim. Prod. 20:401-406.

Collin's English Dictionary, 3rd ed. 1991. Harper Collins Publishers, Glasgow, UK.

Copson, D.A. 1975. Microwave heating. 2nd ed. AVI publishing Co., Connecticut.

Corbit, J.D. 1970. Behavioral regulation of body temperature. In: J.D. Hardy, A.P. Gagge, and J.A.J. Stolwijk, eds. Physiological and behavioral temperature regulation. Charles C. Thomas, Springfield, Ill. Pp. 777-801.

Cramer, H., Rudolf, J., Consbruch, U. and Kendel, K. 1974. On the effect of melatonin on sleep and behaviour in man. Adv. Biochem. Psychopharmacol. 11:187-191.

Cunningham, H.M. and Brisson, G.J. 1955. The utilization of lard by baby pigs. Can. J. Agr. Sci. 35:371-376.

Curtis, S.E. 1983. Environmental Management in Animal Agriculture. Iowa State University Press, Ames, Iowa. P.59-70.

Czeisler, C. A., Allan, J. S., Strogatz, S. H., Ronda, J. M., Sanchez, R., Rios, C.D., Freitag, W. O., Richardson, G. S. and Kronauer, R. E. 1986. Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. Science 233:667-671.

Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N. and Ronda, J. M. 1989. Bright light induction of strong (type O) resetting of the human circadian pacemaker. Science 244:1328-1333.

Czeisler, C.A., Richardson, G.S., Zimmerman, J.C., Moore-Ede, M.C. and Weitzman, E.D. 1981. Entrainment of human circadian rhythms by light-dark cycles: a reassessment. Photochem. Photobiol. 34:239-247.

D'Andrea, J.A., Gandhi, O.P., Lords, J.L., Durney, C.H., Astle, L., Stensaas, L.J. and Schoenberg, A.A. 1980. Physiological and behavioural effects of prolonged exposure to 915 MHz microwaves. J. Microwave Power 15:123-135.

D'Andrea, J.A., Gandhi, O.P., Lords, J.L., Durney, C.H., Johnson, C.C. and Astle, L. 1979. Physiological and behavioural effects of chronic exposure to 2450 MHz microwaves. J. Microwave Power 14:351-362.

DeBoer, H. and Hacker, R.R. 1987. Circadian rhythm of melatonin, thyroxine, and prolactin in sows. Can. J. Anim. Sci. 66:1189 (Abstract).

DeLorge, J. O. 1976. The effects of microwave radiation on behaviour and temperature in rhesus monkeys. In: C. C. Johnson and M. L. Shore, eds. Biological effects of electromagnetic waves. Selected papers of the USNC/URSI annual meeting, vol. 1. Public Health Service, Rockland, Md. P.158-174.

DeLorge, J.O. 1978. Disruption of behaviour in mammals of three different sizes exposed to microwaves: extrapolation to larger mammals. In: Stuchley, S.S., ed. Symposium on Electromagnetic Fields in Biological Systems. Ottawa.

Dement, W.C. 1958. The occurrence of low voltage, fast electroencephalogram patterns during behavioral sleep in the cat. Electroencephalogr. Clin. Neurophysiol. 10:291-296.

Dement, W.C., Mitler, M.M. and Henriksen, S.J. 1972. Sleep changes during chronic administration of parachlorophenylalanine. Rev. Can. Biol. 31:239-246.

Denoyer, M., Sallanon, M., Kitahama, K., Aubert, C. and Jouvet, M. 1989. Reversibility of parachlorophenylalanine induced insomnia by intrahypothalamic microinjection of L-5-hydroxytryptophan. Neurosci. 28:83-94.

Dewey, C.E., Martin, S.W., Friendship, R.M. and Wilson, M.R. 1994. The effects on litter size of previous lactation length and previous weaning-to-conception interval in Ontario swine. Prev. Vet. Med. 18:213-223.

Diekman, M. A., Clapper, J. A., Green, M. L. and Stouffer, D. K.1991. Reduction in age of puberty in gilts consuming melatonin during decreasing or increasing daylength. J. Anim. Sci. 69:2524-2531.

Dollins, A. B., Zhadanova, I. V., Wurtman, R. J., Lynch, H. J. and Deng, M. H. 1994. Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. Proc. Natl. Acad. Sci. USA 91:1824-1828.

Donham, K.J. 1991. Association of environmental air contaminants with disease and productivity in swine. Am. J. Vet. Res. 52:1723-1730.

English, P.R., Fowler, V.R., Baxter, S. and Smith, B. 1988. The growing and finishing pig: improving efficiency. Farming Press, Ipswich, UK.

English, P.R., Smith, W.J. and MacLean, A. 1977. The sow - improving her efficiency. Farming Press Ltd., Ipswich, UK.

Evans, F.D., Christopherson, R.J. and Aherne, F.X. 1988. Development of the circadian rhythm of cortisol in the gilt from weaning until puberty. Can. J. Anim. Sci. 68:1105-1111.

Ewebank, R. 1969. Social behaviour and intensive animal production. Vet. Rec., 85:183-186.

Ewebank, R. and Bryant, M.J. 1972. Aggressive behaviour amongst groups of domesticated pigs kept at various stocking rates. Anim. Behav. 20:21-28.

Ewebank, R. and Meese, G.B. 1971. Aggressive behaviour in groups of domesticated pigs on removal and return of individuals. Anim. Prod. 13:685-693.

Favale, E., Loeb, E., Rossi, G.F. and Sacco, G. 1961. EEG synchronization and behavioural signs of sleep following low frequency stimulation of the brainstem reticular formation. Arch. Ital. Biol. 99:1-22.

Foote, K.D., Bate, L.A. and Donald, A.W. 1996. Activity level and performance of weaner pigs exposed to 915 MHz microwave radiation. Can. J. Anim. Sci. 76:183-188.

Fraser, D. 1974. The behaviour of growing pigs during experimental social encounters. J. Agric. Sci. Camb. 82:147-163.

Freemon, F.R. 1972. Sleep Research. Charles C. Thomas, Springfield, Ill.

Frey, A.H. 1962. Human auditory system response to modulated Electromagnetic energy. J. Appl. Physiol. 17:689-692.

Frey, A.H., Feld, S.R. and Frey, B. 1975. Neural function and behavior: defining the relationship. Ann. N.Y. Acad. Sci. 247:433-439.

Friend, T.H., Knabe, D.A. and Tanksley, T.D. 1983. Behaviour and performance of pigs grouped by three different methods at weaning. J. Anim. Sci. 57:1406-1411.

Friendship, R.M. 1987. A reduction in litter size on an Ontario swine farm. Can. Vet. J. 28:120-123.

Frobish, L.T., Hays, V.W., Speer, V.C. and Ewan, R.C. 1969. Effect of diet form and emulsifying agents on fat utilization by young pigs. J. Anim. Sci. 29:320-324.

Gage, M.I., Berman, E. and Kinn, J.B. 1979. Videotape observation of rats and mice during an exposure to 2450 MHz microwave radiation. Radio Science 14:227-232.

Galloway, W.D. 1975. Microwave dose-response relationships on two behavioural tasks. Ann. N.Y. Acad. Sci. 247:410-416.

Garcia-Arraras, J.E. and Pappenheimer, J.R. 1983. Site of action of sleep-inducing muramyl peptide isolated from human urine: microinjection studies in rabbit brains. J. Neurophysiol. 49:528-533.

Garrett, W.N., Bond. T.E. and Kelly, C.F. 1960. Environmental comparisons of swine performance as affected by shaded and unshaded wallows. J. Anim. Sci. 19:921-925.

Gilliland, M.A., Bergmann, B.M. and Rechtschaffen, A. 1989. Sleep deprivation in the rat: VIII. High EEG amplitude sleep deprivation. Sleep 12:53-59.

Griffith, M. K. and Minton, J. E. 1992. Effect of light intensity on circadian profiles of melatonin, prolactin, ACTH, and cortisol in pigs. J. Anim. Sci. 70:492-498.

Guillet, R., Lotz, W.G. and Michaelson, S.M. 1975. Time course of adrenal response in microwave-exposed rats. In: Proc. Annu. Meeting USRI, Boulder, Co. Oct. 20-23. P.316.

Hafez, E. S. E. and Signoret, J. P. 1969. The behaviour of swine. In: Hafez, E. S. E., ed. Behaviour of domestic animals, 2nd ed. Baillière, Tindall and Cassell, London, England.

Halberg, F. 1969. Chronibiology. Ann. Rev. Physiol. 31:675-725.

Hammel, H.T., Jackson, D.C., Stolwijk, J.A.J., Hardy, J.D. and Strømme, S.B. 1963. Temperature regulation by hypothalamic proportional control with an adjustable set point. J. Appl. Physiol. 18:1146-1154.

Hampson, D.J. 1986. Alterations in piglet small intestinal structure at weaning. Res. Vet. Sci. 40:32-40.

Hampson, D.J. and Kidder, D.E. 1986. Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. Res. Vet. Sci. 40:24-31.

Hayaishi, O. 1988. Sleep-wake regulation by prostaglandins D_2 and E_2. J. Biol. Chem. 263:14593-14596.

Hazlerigg, D.G., Gonzalez-Brito, A., Lawson, W., et al. 1993. Prolonged exposure to melatonin leads to time dependent sensitizaiton of adenylate cyclase and down regulates melatonin receptors in pars tuberalis cells from ovine pituitary. Endocrinology 132:285-292.

Hedlund, L., Lischko, M.M., Rollag, M.D. and Niswender, G.D. 1977. Melatonin: Daily cycle in plasma and cerebrospinal fluid of calves. Science 195:686-687

Heitman, H. and Hughes, E.H. 1949. The effects of air temperature and relative humidity on the physiological well being of swine. J. Anim. Sci. 8:171-181.

Heitman, H., Hahn, L., Bond, T.E. and Kelly, C.F. 1962. The effects of modified summer environment on swine behaviour. Anim. Behav. 10:15-19.

Henken, A.M., Brandsma, H.A., Van der Hel, W. and Verstegen, M.W.A. 1991. Heat balance characteristics of limit-fed growing pigs of several breeds kept in groups at and below thermal neutrality. J. Anim. Sci. 69:2434-2442.

Hernández Peón, R. 1965. Central neurohumoral transmission in sleep and wakefulness. In: K. Akert, C. Bally, and J. P. Schade, eds. Progress in brain research, vol. 18: Sleep mechanisms. Elsevier Publishing Co., Amsterdam. Pp.96-117.

Hess, W.R. 1954. The diencephalic sleep centre. In: Adrian, E.D., Bremer, F., Jasper, H.H., and Delafresnaye, J.R., eds. Brain Mechanisms and Consciousness. Thomas Springfield, Ill. Pp. 117-125.

Hishikawa, Y., Cramer, H. and Kuhlo, W. 1969. Natural and melatonin-induced sleep in young chickens - A behavioural and electrographic study. Exp. Brain Res. 7:84-94.

Hjeresen, D.L., Doctor, S.K. and Shelton, R.L. 1979. Shuttlebox side preference as mediated by pulsed microwave and conventional auditory cues. In: Proceedings of Symposium on Electromagnetic Fields in Biological Systems, International Microwave Power Institute, 78CHI438-1 MTT, Ottawa, Pp.194-214.

Hunt, E.L., King, N.W. and Phillips, R.D. 1975. Behavioral effects of pulsed microwave radiation. Ann. N.Y. Acad. Sci. 247:440-453.

Ibuka, N., Inouye, S.I.T. and Kawamura, H. 1977. Analysis of sleep-wakefulness rhythms in male rats after suprachiasmatic nuclear lesions and ocular enucleation. Brain Res. 122:33-47.

Ibuka, N. and Kawamura, H. 1975. Loss of circadian rhythm in sleep-wakefulness cycle in the rat by suprachiasmatic nucleus lesions. Brain Res. 96:76-81.

Illnerova, H., Zvolsky, P. and Vanecek, J. 1985. The circadian rhythm in plasma melatonin concentration of the urbanised man, the effect of summer and winter time. Brain Res. 328:186-189.

Inouye, S.T. and Kawamura, H. 1979. Persistence of circadian rhythmicity in mammalian hypothalamic "island" containing the suprachiasmatic nucleus. Proc. Natl. Acad. Sci. U.S.A. 76:5962-5966.

IRPA. 1988. International Radiational Protection Association. Guidelines on limits of exposure to radiofrequency electromagnetic fields in the frequency range from 100 MHz to 300 GHz. Health Phys. 54:115-123.

Jensen, P. 1982. An analysis of agonistic interaction patterns in group-housed dry sows: Aggression regulation through an "avoidance order." Appl. Anim. Ethol. 9:47-61.

Jensen, P. 1986. Observations on the maternal behaviour of free-ranging domestic pigs. Appl. Anim. Behav. Sci. 16:131-142.

Johnson, C.C. and Guy, A.W. 1972. Nonionizing electromagnetic wave effects in biological materials and systems. Proc. IEEE 60:692-718.

Johnson, R.B., Myers, D.E., Guy, A.W., Lovely, R.H. and Galambos, R. 1976. Discriminative control of appetitive behaviour by pulse microwave radiation in rats. In: Biological effects of Electromagnetic waves. Selected papers of the USNC/URSI Annual Meetings, Boulder, Colo., Oct 20-23, 1976, HEW Pub. No.(FDA) 77-8010, 1, Pp.238-247.

Jouvet, M. 1972. The role of monoamines and acetylocholine-containing neurons in the regulation of the sleep-waking cycle. Ergeb. Physiol. 64:166-307.

Jouvet, M. 1988. The regulation of paradoxical sleep by the hypothalamo-hypophysis. Arch. Ital. Biol. 126:259-274.

Jouvet, M., Bobillier, P., Pujol, J.F. and Renault, J. 1966. Effects des lésions du système du raphé sur le sommeil et la sérotonine cérébrale. C.R. Soc. Biol. 160:2343-2346.

Jouvet, M. and Renault, J. 1966. Insomnie persistante après lésions des noyaux du raphé chez le chat. C.R. Soc. Biol. 160:1461-1465.

Kaitin, K.I. 1984. Preoptic area unit activity during sleep and wakefulness in the cat. Exp. Neurol. 83:347-357.

Kato, M., Honma, K. I., Shigemitsu, T. and Shiga, Y. 1993. Effects of exposure to a circularly polarized 50-Hz magnetic field on plasma and pineal melatonin levels in rats. Bioelectromagnetics 14:97-106.

Kato, M., Honma, K., Shigemitsu, T. and Shiga, Y. 1994a. Circularly-polarized, 50-Hz, magnetic field exposure reduced pineal gland and blood melatonin concentrations of Long-Evans rats. Neurosci. Lett. 166:59-62.

Kato, M., Honma, K., Shigemitsu, T. and Shiga, Y. 1994b. Horizontal or vertical 50-Hz, 1-μT magnetic fields have no effect on pineal gland or plasma melatonin concentration of albino rats. Neurosci. Lett. 168:205-208.

Kelly, D.D. 1991. Sleep and Dreaming. In: Kandel, E.R., Schwartz, J.H., and Jessell, T.M., eds. Principles of Neural Science, 3rd ed. Elsevier Science Publishing Co., Inc. New York, NY. Pp.792-803.

Klein, D. C. and Moore, R. Y. 1979. Pineal N-acetyltransferase and hydroxyindole-O-methyl transferase: control by the retinohypothalamic tract and the suprachiasmatic nucleus. Brain Res. 174:245-262.

Kopin, I.J., Pare, C.M.B., Axelrod, J. and Weissbach, H. 1961. The fate of melatonin in animals. J. Biol. Chem. 236:3072-3075.

Korbel, S.F. 1969. Behavioural effects of low intensity UHF radiation. In: Biological Effects and Health Implications of Microwave Radiation. Symp. Proc. Med. College of Virginia, Richmond, Va., Rep. RRH/DBE 70-2. Pp.180-184.

Korbel, S.F. and Thompson, W.D. 1965. Behavioural effects of stimulation by UHF radio fields. Psychological Reps. 17:595-602.

Krueger, J.M., Obal, F. Jr., Johanssen, L., Cady, A.B. and Toth, L. 1989. In: Slow Wave Sleep: Physiological, Pathophysiological and Functional Aspects. Wauguier, A., Dugovic, C. and Radulovacki, M., eds. Raven press. Pp75-90.

Kurz, J.C. and Marchinton, R.L. 1972. Radiotelemetry studies of feral hogs in South Carolina. J. wildl. Mgmt. 36:1240-1248.

Kveder, S. and McIsaac, W.M. 1961. The metabolism of melatonin (N-acetyl-5-methoxytryptamine) and 5-methoxytryptamine. J. Biol. Chem. 236:3214.

Launay, J.M., Lemaitre, B. and Husson, H.P. 1982. Melatonin synthesis by rabbit platelets. Life Sci. 31:1487-1494.

Leibrandt, V.D., Ewan, R.C., Speer, V.C. and Zimmerman, D.R. 1975. Effect of weaning and age at weaning on baby pig performance. J. Anim. Sci. 40:1077-1080.

Lerner, A.B., Case, J.D., Takahashi, Y., Lee, T.H. and Mori, W. 1958. Isolation of melatonin, pineal factor that lightens melanocytes. J. Am. Chem. Soc. 80:2587.

L'Hermite-Baleriaux, M., Van Coevorden, A., Laurent, E., Casteels, S., Kerkhofs, M., L'Hermite, M., Mockel, J. and Van Cauter, E. 1989. Melatonin circadian rhythm and sleep in aging. In: Reiter, R.J., and Pang, S.F., eds. Advances in pineal research. John Libbey & Company, Ltd., London, Eng. Pp.311-314.

Lin, J.C. 1978. Microwave auditory effects and applications. Springfield, Ill., Charles C. Thomas, Publisher.

Lin, J.C., Guy, A.W., and Cladwell, L.R. 1977. Thermographic and behavioral studies of rats in the near field of 918 MHz radiations. IEEE Trans. MTT 25:833-836.

Lloyd, L.E., Crampton, E.W. and MacKay, V.G. 1957. The digestibility of ration nutrients by three- vs. seven-week old pigs. J. Anim. Sci. 16:383-388.

Lopez, J., Jesse, G.W., Becker, B.A. and Ellersieck, M.R. 1991a. Effects of temperature on the performance of finishing swine: I. Effects of a hot, diurnal temperature on average daily gain, feed intake, and feed efficiency. J. Anim. Sci. 69:1843-1849.

Lopez, J., Jesse, G.W., Becker, B.A. and Ellersieck, M.R. 1991b. Effects of temperature on the performance of finishing swine: II. Effects of a cold, diurnal temperature on average daily gain, feed intake, and feed efficiency. J. Anim. Sci. 69:1850-1855.

Lotz, W.G., and Michaelson, S.M. 1979. Effects of hypophysectomy and dexamethasone on the rat's adrenal response to microwave irradiation. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 47:1284-1288.

Lotz, W.G. and Michaelson, S.M. 1978. Temperature and corticosterone relationships in microwave-exposed rats. J. Appl. Physiol. 44:438-445.

Lu, S.T., Lebda, N.A., Michaelson, S.M., Pettit, S. and Rivera, D. 1977. Thermal and endocrinological effects of protracted irradiation of rats by 2450 MHz microwaves. Radio Sci. 12:147-156.

Lu, S.T., Lebda, N.A., Pettit, S. and Michaelson, S.M. 1985. The relationship of decreased serum thyrotropin and increased colonic temperature in rats exposed to microwaves. Radiation Research, 104:365-386.

Lu, S.T., Lotz, W.G. and Michaelson, S.M. 1980. Advances in microwave-induced neuroendocrine effects: The concept of stress. Proc. IEEE 68:73-77.

Lucas, I.A.M., Calder, A.F.C. and Smith, H. 1959. The early weaning of pigs. VI. The effects of early weaning and various growth curves before 50 pounds live weight upon subsequent performance and carcass quality. J. Agr. Res. 53:136-144.

Lynch, H.J. 1971. Diurnal oscillations in pineal melatonin content. Life Sci. 10:791-795.

Magnes, J., Moruzzi, G. and Pompeiano, O. 1961. Synchronization of the EEG produced by low-frequency electrical stimulation of the region of the solitary tract. Arch. Ital. Biol. 99:33-67.

Mallick, B.N., Chhina, G.S., Sundaram, K.R., Singh, B. and Mohan Kumar, V.M. 1983. Activity of preoptic neurons durng synchronization and desynchronization. Exp. Neurol. 81:586-597.

Marczynski, T.J., Yamaguchi, N., Ling, G.M. and Grodzinska, L. 1964. Sleep induced by the administration of melatonin (5-methoxy-N-acetyltryptamine) to hypothalamus in unrestrained cats. Experientia 20:435-437.

Maritime Electric Rates Schedule and Policies Manual. Aug. 16, 1999.

Martin, J.B. 1973. Neural regulation of growth hormone secretion. New Eng. J. Med. 288:1384-1393.

McBride, G. 1963. The teat order and communication in young pigs. Anim. Behav. 11:53-56.

McBride, G., James, J.W. and Hodgens, N. 1964. Social behaviour of domestic animals. IV. Growing pigs. Anim. Prod. 6:129-140.

McConnell, S.J. and Ellendorff, F. 1987. Absence of nocturnal plasma melatonin surge under long and short artificial photoperiods in the domestic sow. J. Pineal Res. 4:201-210.

McCracken, K.J. and Caldwell, B.J. 1980. Studies on diurnal variations of heat production and the effective lower critical temperature of early-weaned pigs under commercial conditions of feeding and management. Br. J. Nutr. 43:321-328.

McGinty, D.J., Harper, R.M. and Fairbanks, M.K. 1973. 5-HT containing neurons: unit activity in behaving cats. In: Barchas, J. and Usdin, E., eds. Serotonin and Behavior. Academic Press, New York. Pp. 267-280.

McGinty, D. and Szymusiak, R. 1990. Keeping cool: a hypothesis about the mechanisms and functions of slow wave sleep. Trends Neurosci. 13:155-226.

McLagan, J.R. and Thompson, W. 1950. Effective temperature as a measure of environmental conditions for pigs. J. Agric. Sci., Camb. 40:367-374.

Meese, G.B. and Ewebank, R. 1973. The establishment and nature of the dominance hierarchy in the domesticated pig. Anim. Behav. 21:326-334.

Merritt, J.H., Chamness, A.F. and Allen, S.J. 1978. Studies on blood-brain barrier permeability after microwave-radiation. Radiation and Environmental Biophysics 15:367-377.

Meunier-Salaün, M.C., Monnier, M., Colléaux, Y., Sève, B. and Henry, Y. 1991. Impact of dietary tryptophan and behavioural type on behaviour, plasma cortisol, and brain metabolites of young pigs. J. Anim. Sci. 69:3689-3698.

Michaelson, S.M. Houk, W.M., Lebda, N.J.A., Lu, S.T. and Magin, R.L. 1975. Biochemical and neuroendocrine aspects of exposure to microwaves. Ann. N.Y. Acad. Sci. 247:21-44.

Michaelson, S.M. and Lin, J.C. 1987. Biological effects and health implications of radiofrequency radiation. Plenum Press, New York, NY.

Minton, J.E. and Cash, W.C. 1990. Effect of cranial sympathectomy on circadian rhythms of cortisol, adrenocorticotropic hormone and melatonin in boars. J. Anim. Sci. 68:4277-4284.

Minton, J.E., Davis, D.L. and Stevenson, J.S. 1989. Contribution of the photoperiod to circadian variations in serum cortisol and melatonin in boars. Domest. Anim. Endocrinol. 6:177-181.

Mitchell, D.S., Switzer, W.G. and Bronaugh, E.L. 1977. Hyperactivity and disruption of operant behaviour in rats after multiple exposures to microwave radiation. Radio Science 12:263-271.

Mohan Kumar, V.M., Mallick, B.N., Chhina, G.S. and Singh, B. 1985. Alterations in preoptic unit activity on stimulation of caudal brain stem EEG-synchronizing structures. Exp. Neurol. 89:304-313.

Moore, R.Y. and Eichler, V.B. 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic nucleus lesions in the rat. Brain Res. 42:201-206.

Moore, R. Y. and Klein, D. C. 1974. Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. Brain Res. 71:17-33.

Moore, R.Y. and Lenn, N.J. 1972. A retinohypothalamic projection in the rat. J. Comp. Neurol. 146:1-14.

Moore-Ede, M.C., Czeisler, C.A. and Richardson, G.S. 1983. Circadian timekeeping in health and disease. Basic properties of circadian pacemakers. N. Engl. J. Med. 309:469-476.

Morairty, S.R., Szymusiak, R., Thomson, D. and McGinty, D.J. 1993. Selective increases in non-rapid eye movement sleep following whole body heating in rats. Brain Res. 617:10-16.

Morgan, P.J. and Williams, L.M. 1989. Central melatonin receptors: implications for a mode of action. Experientia 45:955-965.

Moriyama, E., Salcman, M. and Broadwell, R.D. 1991. Blood-brain barrier alteration after microwave-induced hyperthermia is purely a thermal effect: I. Temperature and power measurements. Surg. Neurol. 35:177-182.

Morrison, W. D., Amyot, E., McMillan, I. and Otten, L. 1987. Performance of male broiler chicks exposed to heat from infrared or microwave sources. Poult. Sci. 66:1762-1765.

Morrison, W.D., McMillan, I., Bate, L.A. and Otten, L. 1986. Behavioural observations and operant procedures using microwaves as a heat source for young chicks. Poult.Sci. 65:1516-1521.

Moruzzi, G. 1972. The sleep-waking cycle. Ergeb. Physiol. 64:1-165.

Moruzzi, G. and Magoun, H.W. 1949. Brainstem reticular formation and activation of the EEG. EEG. Clin. Neurophysiol. 1:455-473.

Mount, L.E. 1960. The influence of huddling and body size on the metabolic rate of the young pig. J. Agric. Sci. Camb. 55:101-105.

Mount, L.E. 1966. The effect of wind speed on heat production in the new born pig. Q. J. Exp. Physiol. 51:18-26.

Mount. L.E. 1967. The heat loss from new born pigs to the floor. Res. Vet. Sci. 8:175-186.

Mount, L.E. 1968. The climatic physiology of the pig. Edward Arnold Publishers Ltd. London, England.

Murgatroyd, D. and Hardy, J.D. 1970. Temperature regulation by operant behavior in the rat. In: J.D. Hardy, A.P. Gagge, and J.A.J. Stolwijk, eds. Physiological and behavioral temperature regulation. Charles C. Thomas, Springfield, Ill.

Myers, B.L. and Badia, P. 1993. Immediate effects of different light intensities on body temperature and alertness. Physiol. Behav. 54:199-202.

Nelssen, J.L., Goodband, R.D., Dritz, S.S. and Tokach, M.D. 1995. The effect of weaning age on nursery pig feeding behavior and growth performance. Advances in Pork Production 6:95-112.

Ogawa, Y. and Kawamura, H. 1988. Increase of multiple unit activity during slow wave sleep in the cat preoptic area. Brain Res. Bull. 20:897-902.

Olfert, E. D., Cross, B. M. and McWilliam, A. A. 1993. Guide to the care and use of experimental animals, Vol. 1. 2nd ed. Canadian Council on Animal Care, Ottawa, Ont.

Ookawa, T. 1972. Avian wakefulness and sleep on the basis of recent electroencephalographic observations. Poult. Sci. 51:1565-1574.

Oswald, I. 1980. Sleep as a restroative process: Human clues. Prog. Brain Res. 53:279-288.

Ozaki, Y., Lynch, H.J. and Wurtman, R.J. 1976. Melatonin in rat pineal, plasma, and urine: 24-hour rhythmicity and effect of chlorpromazine. Endocrinology 98:1418-1424.

Pang, S.F., Brown, G.M., Grota, L.J., et al. 1977. Determination of N-acetylserotonin and melatonin activities in the pineal gland, retina, and Harderian gland, brain and serum of rats and chickens. Neuroendocrinol. 23:1-13.

Parker, L.N. 1973. Thyroid suppression and adrenomedullary activation by low-intensity microwave radiation. Am. J. Physiol. 224:1388-1390.

Petitjean, F., Buda, C., Janin, M., Sallanon, M. and Jouvet, M. 1985. Insomnie par administration de parachlorophénylalanine: réversibilité par injection périphérique ou centrale de 5-hydroxytryptophane et de sérotonine. Sleep 8:56-67.

Petrov, I.R. and Syngayevskaya, V.A. 1970. Endocrine glands. In: Petrov, I.R., ed. Influence of Microwave Radiation on the Organism of Man and Animals. Meditsina Press, Leningrad.

Pool, R. 1990. Is there an EMF-cancer connection? Science 249:1096-1098.

Portaluppi, F., Cortelli, P., Avoni, P., Vergnani, L., Maltoni, P., Pavani, A., Sforza, E., Uberti, E.C.D., Gambetti, P. and Lugaresi, E. 1994. Progressive disruption of the circadian rhythm of melatonin in fatal familial insomnia. J. Clin. Endocrinol. Metab. 78:1075-1078.

Porter, P. 1969. Procine colostral IgA and IgM antibodies to *Escherichia coli* and their intestinal absorption by the neonatal piglet. Immunology 17:617-626.

Porter, P. and Hill, I.R. 1970. Serological changes in immunoglobulins IgG, IgA and IgM and *Escherichia coli* antibodies in the young pig. Immunology 18:565-573.

Rafai, P and Fodor, E. 1980. Studies on porcine adrenorcortical function. I. Circadian variations in, and effect of ACTH treatment on, the peripheral plasma cortisol level. Act. Vet. Scand. Sci. Hung. 28:433-441.

Raikhlin, N.T., Kvetnoy, I.M. and Tolkachev, V.N. 1975. Melatonin may be synthesized in enterochromaffin cells. Nature 225:344-345.

Rasmussen, O.G., Banks, E.M., Berry, I.H. and Becker, D.E. 1962. Social dominance in gilts. J. Anim. Sci. 21:520-522.

Rechtschaffen, A., Gilliland, M.A., Bergmann, B.M. and Winter, J.B. 1983. Physiological correlates of prolonged sleep deprivation in rats. Science 221:182-184.

Reece, W.O. 1991. Physiology of Domestic animals. Lea and Febiger, Philadelphia, PA.

Reiter, R.J. 1986. Normal patterns of melatonin levels in the pineal gland and body fluids of humans and experimental animals. J. Neural. Transm. 21 (suppl.) 35-54.

Reiter, R.J. 1987. The melatonin message: duration versus coincidence hypotheses. Life Sci. 40:2119-2131.

Reiter, R.J. 1993. Static and extremely low frequency electromagnetic field exposure: reported effects on the circadian production of melatonin. J. Cell. Biochem. 51:394-403.

Reiter, R.J., Brit, J.H. and Armstrong, J.D. 1987. Absence of a nocturnal rise in either norepinephrine, N-acetyltransferase, hydroxyindole-O-methyl transferase or melatonin in

the pineal gland of the domestic pig kept under natural environmental photoperiods. Neurosci. Lett. 81:171-176.

Repacholi, M. H. 1990. Radiofrequency field exposure standards: Current limits and relevant bioeffects data. In: O. P. Gandhi, ed., Biological Effects and Medical Applications of Electromagnetic Energy. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Reppert, S.M., Weaver, D.R., Rivkees, S.A. and Stopa, E.G. 1988. Putative melatonin receptors in a human biological clock. Science 242:78-81.

Richter, C.P. 1967. Sleep and activity: their relation to the 24-hour clock. Res. Publ. Assoc. Res. Nerv. Ment. Dis. 45:8-27.

Robert, S. and Dallaire, A. 1986. Polygraphic analysis of the slep-wake states and the REM Sleep periodicity in domestic pigs (*Sus scrofa*). Physiol. Behav. 37:289-293.

Roberti, B., Heebels, G.H., Hendricx, J.C.M., DeGreef, A.H.A.M. and Wolthuis, O.L. 1975. Preliminary investigations of the effects of low-level microwave radiation on spontaneous motor acitivity in rats. Ann. N.Y. Acad. Sci. 247:417-424.

Rogers, W.R., Reiter, R.J., Smith, H.D. and Barlow-Walden. L. 1995. Rapid onset/offset variably-scheduled, 60 Hz electric and magnetic field exposure reduces nocturnal serum melatonin concentration in nonhuman primates. Bioelectromagnetics suppl. 3:119-122.

Rollag, M.D. and Niswender, G.D. 1976. Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. Endocrinology 98:482-489.

Romijin, H.J. 1978. The Pineal, A tranquilizing organ? Life Sci. 23:2257-2274.

Ruckebusch, Y. 1972. The relevance of drowsiness in the circadian cycle of farm animals. Anim. Behav. 20:637-643.

Rushen, J. 1984. Stereotyped behaviour, adjunctive drinking and the feeding periods of tethered sows. Anim. Behav. 32:1059-1067.

Sakaguchi, S., Glotzbach, S.F. and Heller, H.C. 1979. Influence of hypothalamic and ambient temperatures on sleep in kangaroo rats. Am. J. Physiol. 237:R80-R88.

Saleh, M.A. and Winget, C.M. 1977. Effect of suprachiasmatic lesions on diurnal heart rate rhythm in the rat. Physiol. Behav. 19:561-564.

SAS Institute, Inc. 1985. SAS/STAT™ Guide for personal computers, Version 6 Edition. SAS Institute Inc., Cary, NC.

SAS Institute, Inc. 1997. SAS/STAT™ Guide for personal computers, Version 7 Edition. SAS Institute Inc., Cary, NC.

Schally, A.V., Arimura, A. and Kastin. A.J. 1973. Hypothalamic regulatory hormones. Science 179:341-350.

Schwan, H. 1958. Molecular response characteristics to ultra-high frequency fields. In: Proc. 2nd Tri-Serv. Conf. on Biol. Effects of Microwave Energy (Rome, N.Y.) P.33.

Scott, A. W. 1993. Understanding Microwaves. John Wiley and Sons, Inc., New York, NY.

Servantie, B. M. and Gillard, J. 1983. Biological effects and dosimetry of non-ionizing radiation. In: Grendolfo, M., Michaelson, S.M. and Rindi, A., eds. Radiofrequency and microwave energy. Plenum Press, New York, NY. Pp 393-410.

Serway, R.A. 1986. Physics for Scientists and Engineers. 2nd ed. Saunders College Publishing, New York, NY.

Shanahan, T.L. and Czeisler, C.A. 1991. Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. J. Clin. Endocrinol. Metab. 73:227-235.

Shanawany, M. M. 1990. Heating effect of microwave radiation in broilers. Br. Poult. Sci. 31: 361-369.

Signoret, J.P., Baldwin, B.A., Fraser, D. and Hafez, E.S.E. 1975. The behaviour of swine. In: Hafez, E.S.E., ed. The Behaviour of Domestic Animals, 3rd ed. Ballière and Tindall, London, England. Pp. 295-329.

Stanton, H.C. and Mueller, R.L. 1976. Sympathoadrenal neurochemistry and early weaning of swine. Am. J. Vet. Res. 37:779-783.

Stephan, F. K. and Nuñez, A. A. 1977. Elimination of circadian rhythms in drinking, activity, sleep and temperature by isolation of the suprachiasmatic nuclei. Behav. Biol. 20: 1-16.

Stephan, F.K. and Zucker, I. 1972. Circadian rhythms in drinking behaviour and locomotor activity of rats are eliminated by hypothalamic lesions. Proc. Natl. Acad. Sci. U.S.A. 69:1583-1586.

Stephens, D.B. 1971. The metabolic rates of newborn pigs in relation to floor insulation and ambient temperature. Anim. Prod. 13:303-313.

Sterman, M.B. and Clemente, C.D. 1962a. Forebrain inhibitory mechanisms: Cortical synchronization induced by basal forebrain stimulation. Exp. Neurol. 6:91-102.

Sterman, M.B. and Clemente, C.D. 1962b. Forebrain inhibitory mechanisms: Sleep patterns induced by basal forebrain stimulation in the behaving cat. Exp. Neurol. 6:103-117.

Svajgr, A.J., Hays, V.W., Cromwell, G.L. and Dutt, R.H. 1974. Effect of lactation duration on reproductive performance of sows. J. Anim. Sci. 38:100-105.

Szymusiak, R., Danowski, J. and McGinty, D. 1991. Exposure to heat restores sleep in cats with preoptic/anterior hypothalamic cell loss. Brain Res. 541:134-138.

Szymusiak, R. and McGinty, D.J. 1986. Sleep-related neuronal discharge in the basal forebrain of cats. Brain Res. 370:82-92.

Tamarkin, L., Reppert, S.M. and Klein, D.C. 1979. Regulation of pineal melatonin in the Syrian hamster. Endocrinology 104:385-389.

Taylor, L.S. 1981. THe mechanisms of athermal microwave biological effects. Bioelectromagnetics 2:259-267.

Terlouw, E.M., Lawrence, A.B. and Illius, A.W. 1991. Relationship between agonistic behaviour and propensity to develop excessive drinking and chain manipulation in pigs. Physiol. Behav. 50:493-498.

Thomas, J.R., Finch, E.D., Fulk, D.W. and Burch, L.S. 1975. Effects of low-level microwave radiation on behavioural baselines. Ann. N.Y. Acad. Sci. 247:425-432.

Ursin, R. 1970. Sleep stage relations within the sleep cycles of the cat. Brain Res. 20:91-97.

Van Putten, G. 1969. An investigation into tail biting among fattening pigs. Br. Vet. J. 125:511-517.

Van Twyver, H. 1969. Sleep patterns of five rodent species. Physiol. Behav. 4:901-908.

Verhagen, J.M.F., Kloosterman, A.M., Slijkhuis, A. and Verstegen, M.W.A. 1987. Effect of ambient temperature on energy metabolism in growing pigs. Anim. Prod. 44:427-433.

Verstegen, M.W.A., Close, W.H., Start, I.B. and Mount, L.E. 1973. The effects of environmental temperature and plane of nutrition on heat loss, energy retention and deposition of protein and fat in groups of growing pigs. Br. J. Nutr. 30:21-35.

Vertes, R.P. 1990. Brainstem mechanisms of slow-wave sleep and REM sleep. In: Klemm, W.R. and Vertes, R.P., eds. Brainstem Mechanisms of Behaviour. John Wiley and Sons, Inc., New York, NY. Pp.535-583.

Vollrath, L., Semm, P. and Gammel, G. 1980. Sleep induction by intranasal application of melatonin. In: Birau, N. and Schloot, W., eds. Advances in the Biosciences. Proceedings of the International Symposium on Melatonin 29:327-329.

Walton, P.E. and Etherton, T.D. 1986. Stimulation of lipogenesis by insulin in swine adipose tissue: antagonism by porcine growth hormone. J. Anim. Sci. 62:1584-1595.

Webb, W.B. 1971. Sleep as a biorhythm. In: Colquhour, W.P., ed. Biological Rhythms and Human Performance. Academic Press, London, Eng.

Webb, W.B. and Dube, M.G. 1981. Temporal characteristics of sleep. In: Aschoff, J., ed. Handbook of Behavioural Neurobiology. Vol. 4, Biological Rhythms. Plenum Press, New York, NY. Pp.499-522.

Weissbach, H., Redfield, B.G. and Axelrod, J. 1961. The enzymatic acetylation of serotonin and other naturally occurring amines. Biochim. Biophys. Acta. (Amst.) 54:190-192.

Wever, R.A. 1989. Light effects on human circadian rhythms: a review of recent andechs experiments. J. Biol. Rhythms 4:161-185.

Whang, K.Y. and Easter, R.A. 1995. Effect of starter feeding on growth performance and lean body mass at market weight in two different swine genotypes. J. Anim. Sci. 73(suppl.1):83 (Abstract).

Whipp, S,C., Wood, R.L. and Lyon, N.C. 1970. Diurnal variation in concentrations of hydrocortisone in plasma of swine. Am. J. Vet. Res. 31:2105-2107.

Whittemore, C. 1993. The science and practice of pig production. Longman Group UK limited. Essex, England.

Whittemore, C.T., Aumaitre, A. and Williams, I.H. 1978. Growth of body components in young weaned pigs. J. Agric. Sci. 91:681-692.

Widdowson, E.M. 1950. Chemical composition of newly born mammals. Nature 166:626-628.

Wilkinson, M., Arendt, J., Bradtke, J. and De Ziegler, D. 1977. Determination of a dark-induced increase of pineal N-acetyltransferase activity and simultaneous radioimmunoassay

of melatonin in pineal serum and pituitary tissue of the male rat. J. Endocrinol. 72: 243-244.

Williams, W.M., Hoss, W., Formaniak, M. and Michaelson, S.M. 1984. Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. A. Effect on permeability to sodium fluorescein. Brain Research 319:165-170.

Wilson, B.W., Anderson, L.E., Hilton, D.I. and Phillips, R.D. 1981. Chronic exposure to 60 Hz fields: Effects on pineal function in the rat. Bioelectromagnetics 2:371-380.

Wilson, M.R., Friendship, R.M., McMillan, I., Hacker, R.R., Pieper, R. and Swaminathan, S. 1986. A survey of productivity and its component interrelationships in Canadian swine herds. J. Anim. Sci. 62:576-582.

Wilson, M.R. and Gordon, T.P. 1989. Nocturnal changes in serum melatonin during female puberty in rhesus monkeys: A longitudinal study. J. Endocrinol. 121:553-562.

Wilson, R.H. and Leibholz, J. 1981. Digestion in the pig between 7 and 35 days of age. 1. The performance of pigs given milk and soy-bean proteins. Br. J. Nutr. 45:301-319.

Worsaae, H. and Schmidt, M. 1980. Plasma cortisol and behaviour in early weaned piglets. Acta. Vet. Scand. 21:640-657.

Yellon, S.M. and Gottfried, L. 1992. An acute 60 Hz magnetic field expsoure suppresses the nighttime melatonin rhythm in the adult Djungarian hamster in short days. Proc. 1992 Annual Rev. Bioeffects Res., U.S. Department of Energy and EPRI, A-22.

Zernicki, B. 1986. Pretrigeminal preparation. Arch. Ital. Biol. 124:133-196.

Zernicki, B. 1988. Sleep-awaking cycle in brainstem transected animals. In: Oniani, T., ed., Neurobiology of Sleep-Wakefulness Cycle. Metsniereba, Tblisis (USSR). Pp. 103-118.