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PREPRANDIAL AND POSTPRANDIAL CONCENTRATIONS OF SOME METABOLIC HORMONES AND PERFORMANCE IN WEANED PIGLETS FED DIFFERENT DIETS

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Serum concentrations of insulin, cortisol, insulin–like growth factor-1 (IGF-1), thyroxine (T4) and triiodothyronine (T3) were determined before and after feeding weaned piglets fed different diets in two experiments carried out in the winter and summer.

In experiment 1 the standard diet (CP — 20.2%) was replaced with an isocaloric ration containing slightly less crude protein (17.5%; diet 2). Diet 3 (CP — 19.6%) was isonitrogenous with diet 1 but more concentrated. Except for very low preprandial insulin concentrations in most piglets, serum hormone concentrations were within the ranges given by other authors. The slightly better performance of the piglets fed diet 3 was significantly correlated with increased concentrations of T3 and T4. IGF-1 was lower in the piglets fed less protein. Postprandial insulin concentrations were negatively correlated with postprandial cortisol concentrations probably due to differences in the reaction of individual piglets to the overnight fast.

In experiment 2 the fishmeal supplement in the standard diet was replaced by yeast meal. Although the piglets fed the experimental ration performed better, the very low concentrations of thyroid hormones indicated an inadvertent iodine deficiency. Cortisol and insulin concentrations were similar to those found in experiment 1 but IGF-1 values were lower. It was concluded that the diet and season probably combined to allow a lower basal metabolic rate in the piglets in experiment 2 especially those fed diet 2 They were therefore more resistant to the overnight fast, ate more slowly but grew faster than the piglets in experiment 1 because fewer nutrients were used for maintenance requirements.

Key words: Piglets, performance,metabolic hormones, different diets.

INTRODUCTION

During the last twenty five years data on the circulating levels of metabolic hormones and growth factors have been slowly accumulating, together with increasing knowledge about their mechanisms of action in the mammalian organism.

Of all farm animals the complex interrelationships between serum hormone levels and their effects are probably best understood in swine. Thus, Pell and Bates (1990) reported that administration of increasing amounts of growth hormone (GH) progressively decreased feed consumption and adipose tissue deposition but increased protein and mineral accretion. The greatest effect was found in castrated animals, followed by females, while the smallest effect occurred in entire males, which anyhow gave the leanest carcasses and the lowest feed consumption. These effects did not depend on energy intake but the diets had to provide an adequate amount of high quality protein. Campbell (1988) had concluded earlier that the level of endogenous secretion of GH was an important factor limiting lean tissue deposition in pigs fed adequate diets.

It is well known that most of the anabolic actions of GH take place through the mediation of insulin-like growth factor-1 (IGF-1; somatomedin-C), a proinsulin-like molecule synthesised in the liver and many other tissues. The main target tissues for action of this liver derived IGF-1 are bone and muscle, where it triggers anabolic events by activating specific receptors on the cell surface. Since IGF-1 is protected from rapid degradation by binding proteins, serum levels are more stable than the pulsatile pattern observed for GH. Therefore, it should be easier to correlate differences in IGF-1 levels with performance using fewer measurements than is necessary for GH.

In addition, both insulin and corticoid hormone levels have shown positive correlations with the amount of adipose tissue and negative correlations with muscle deposition. Thus, Pandža and coworkers (1975) were able to correlate differences in serum insulin concentration with breed i.e. the normal fasting concentration of serum insulin was significantly lower in a pork breed (Swedish Landrace) than in a fat breed (Mangalitza). Similar differences were established between lean (Yorkshire) and obese (Ossabow) pigs by McCusker and Wangsness (1979) and between Piétrain and German Yorkshire pigs by Ensinger and coworkers (1979). Namely, fasting serum insulin concentrations and the response to feeding were correlated with the genetic origin of the animals. As well as breed differences associated with leanness and obesity, De Wilde (1984) showed that genetically controlled stress susceptibility, as determined by sensitivity to halothane, was also associated with differences in performance and hormone status within a breed (Belgian Landrace). The halothane sensitive animals had a lower response to insulin after a meal, less efficient feed energy conversion but produced leaner carcasses than halothane resistant littermates.

The role of the thyroid hormones as general regulators of metabolic rate should not be ignored either.

With this in mind, serum concentrations of insulin, cortisol, IGF-1, thyroxine (T4) and triiodothyronine (T3) were determined before and after feeding weaned

piglets kept under different dietary regimens, with the aim of gaining a preliminary understanding of factors affecting the balance of metabolic hormones under prevailing conditions of swine production in this locality and their relation to performance.

MATERIALS AND METHODS

The experiments involved female and castrated male piglets (crossbred Swedish Landrace and Yorkshire) kept in groups in mesh-floored pens on the experimental pig farm of the Research Institute for Animal Husbandry in Zemun.

In the first experiment, after weaning at 8 kg body weight, the piglets were fed three different diets. The control group received the standard ration (ME — 13.7 MJ/kg) based on maize grain, wheat, soybean oilmeal and fishmeal which was normally used on the farm. The second group received an isocaloric diet (ME — 13.8 MJ/kg) formulated according to French norms (Rhone-Poulenc, 1989) which are based on the content of metabolisable energy and digestible amino acids. This diet contained no wheat but included a lysine supplement. The crude protein content (17.5%) was lower than in diet 1 (20.2%). The third group was offered a similar diet with 110% of the French norms, which was achieved by adding methionine and oil supplements besides lysine. This diet (19.6% crude protein) was isonitrogenous with diet 1 but was more concentrated (ME — 15.3 MJ/kg). The performance of the 36 piglets included in this experiment and details of the diets have been given earlier (Živković et al.,1992).

Towards the end of the 46 day experiment four piglets of body weight close to the means for the groups (about 25 kg) were separated and placed in individual cages. Blood was taken from the retroorbital sinus at 9 am after a 16 h fast and each piglet was then offered a portion of its respective diet. The time the piglet started to eat was noted and a further blood sample was taken 30 to 45 minutes later. Blood sampling was completed at 10^{40} am. The amount of feed consumed was determined. This experiment was carried out in December 1991 and the ambient temperature in the unit was 20° C.

In the second experiment, which was performed in a similar way, the control group of piglets was fed a standard maize/soybean oilmeal diet containing 4% fishmeal. The second group of piglets received an isonitrogenous, isocaloric diet (ME — 13.5 MJ/kg; CP — 20.2%) in which the fishmeal had been replaced by yeast with methionine and lysine supplements. Blood samping from two female piglets and from two barrows from each group was carried out in July 1992 on two separate days before and after feeding. Thus, the experiment had a 2^4 factorial design.

After separation of the serum by centrifugation, the hormones T3, T4 and cortisol were determined by radioimmunoassay using commercial kits in accordance with the instructions (INEP-Diagnostics, Zemun). Insulin was measured using a homologous radioimmunoassay kit which included porcine insulin standards. IGF-1 was determined after separation from the binding proteins by the method of Daughaday and coworkers (1982) which has been validated for porcine serum (Owens et al., 1990). Briefly, the serum was treated with four

volumes of an acid ethanol mixture, centrifuged after 30 minutes and the supernatant neutralised with Tris. Since porcine IGF-1 has the same amino acid composition as human IGF-1 (Simmen, 1991), the reagents used in the radioimmunoassay were rabbit anti-IGF-1 (UCB-Bioproducts, Belgium) and recombinant human IGF-1 (Serva Feinbiochemica, Germany). The latter substance was labelled with radioactive iodine (125-I) using the chloramine-T method developed for insulin (Nikolić et al., 1989). In the absence of a reference standard the results were expressed as units per litre. The assay was reproducible as indicated by additions of known amounts of standard IGF-1 to serum extracts, extraction of several aliquots of the same serum and progressive dilution of serum extracts with buffer. The intrassay coefficient of variation for four separate extracts of a single serum each determined in duplicate, was 5.5%.

RESULTS

It had been planned to examine four piglets (three barrows and one female) from each group in experiment 1. One piglet from group 2 was inadvertently fed overnight and was therefore excluded; one preprandial blood sample from the control group was lost in the centrifuge and so the results consisted of ten preprandial and eleven postprandial values. The results of the analysis of variance for all the parameters examined in both experiments are given in Table 1.

The results for T3 and T4 are shown in Figure 1 together with those for the eight piglets examined in experiment 2. It can be seen that T4 concentrations differed greatly between the two experiments, being above 40 nmol/L for each piglet in experiment 1 and below 40nmol/L for each piglet in experiment 2. Analytical errors were excluded by determining T4 levels in sera from both experiments in the same test. There was a tendency for T4 levels to be higher in the piglets fed the more concentrated diet 3 than in the piglets from the other groups in experiment 1 ($P<0.05$). Within experiment 2, piglets fed the diet containing yeast instead of fishmeal had very low T4 levels (<20 nmol/L). The difference between the two diets was statistically highly significant ($P<0.001$; Table 1). There were no differences in T4 concentrations in relation to feeding but in experiment 2 the female piglets tended to have higher T4 levels. However, there was a significant treatment/sex interaction in that this difference was only apparent with the standard diet. The small number of animals involved, the low statistical significance (Table 1) and the absence of an effect in experiment 1 suggest that this was probably the result of chance.

Serum concentrations of T3 were also higher in experiment 1 than in experiment 2, although the differences were less pronounced. There were statistically significant differences between postprandial and preprandial values in both experiments ($P<0.05$). No significant effects of diet or sex were apparent in experiment 1 but analysis of variance for experiment 2 showed that all four factors were statistically significant (Table 1). Namely, values were higher on day 2 than day 1, in females than in barrows, and with diet 2 than with diet

1. The mean concentration of serum T3 in experiment 2 (0.50 nmol/L)was much lower than that in experiment 1 (0.93 nmol/L).

Table 1. Analysis of variance for various parameters in piglets

		Experiment 1				Experiment 2			
Parametar	Factor	F	P	LSD	SE	F	P	LSD	SE
Serum T4	Feeding	0.54	NS			0.30	NS		
(nmol/L)	Diet	3.88	<0.05	14.6	11.9	77.37	<0.01	3.9	3.5
	Sex	0.01	NS			7.19	<0.05		
	Day	—				0.87	NS		
Serum T3	Feeding	5.65	<0.05			5.73	<0.05		
(nmol/L)	Diet	1.90	NS	0.33	0.27	4.88	<0.05	0.09	0.08
	Sex	2.46	NS			9.79	<0.01		
	Day	—				12.24	<0.01		
Serum	Feeding	1.09	NS			1.99	NS		
Cortisol	Diet	0.64	NS	75.0	61.3	7.85	<0.05	59.9	54.5
(nmol/L)	Sex	3.06	NS			0.04	NS		
	Day	—				2.77	NS		
Serum	Feeding	30.57	<0.01			8.23	<0.05		
Insulin	Diet	0.04	NS	14.1	13.2	0.08	NS	35.5	32.2
(mIU/L)	Sex	1.49	NS			0.05	NS		
	Day	—				0.06	NS		
Serum	Feeding	2.93	NS			0.19	NS		
IGF-1	Diet	8.83	<0.01	37.4	30.4	1.51	NS	55.4	50.3
(U/L)	Sex	21.81	<0.01			0.55	NS		
	Day	—				5.97	<0.05		
Weight	Diet	2.20	NS			3.63	NS		
gain	Sex	1.17	NS	70.6	40.5	1.59	NS	112	61.6
(g/day)									
Feed	Diet	1.95	NS			3.65	NS		
consumed	Sex	1.79	NS	109	62.5	2.16	NS	94.4	48.1
(g)	Day	—				0.54	NS		
Eating	Diet	2.79	NS			7.20	0.05		
rate	Sex	0.08	NS	3.3	1.9	1.91	NS	2.6	1.3
(g/min)	Day	—				0.32	NS		

There was a highly significant positive correlation between fasting concentrations of T4 and T3 in experiment 1 ($r = 0.886$; $P<0.01$), namely piglets with higher serum T4 also had higher levels of T3 (Figure 4). This relationship was not apparent in experiment 2 ($r = -0.274$).

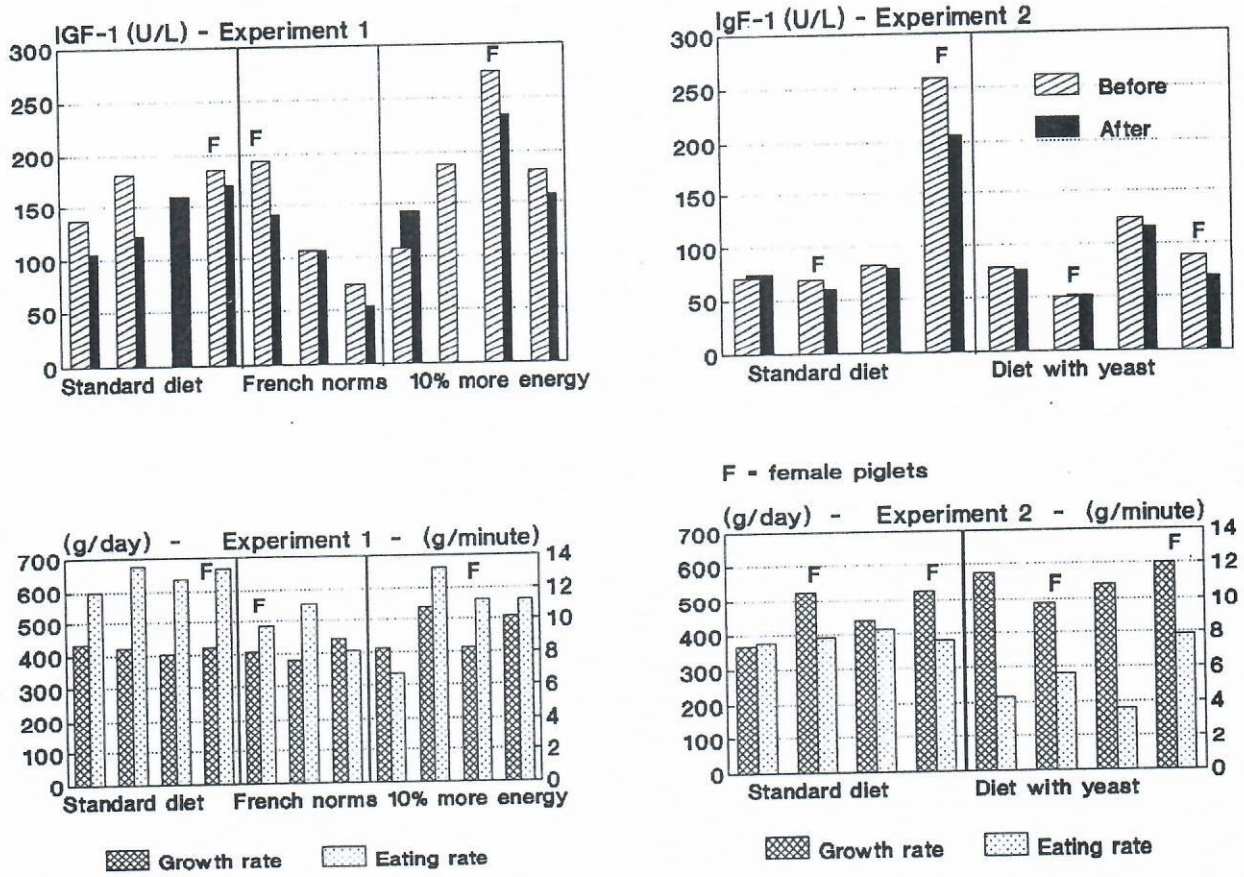

Figure 1. Serum concentrations of T4 and T3 before and 30 to 45 minutes after feeding weaned piglets (barrows and females) different diets.

The values for serum cortisol concentrations found in individual piglets are given in Figure 2. Levels varied widely within both experiments in the range from 14 nmol/L to 260 nmol/L. Both increases and decreases were noted after feeding the piglets. No statistically significant differences in relation to feeding or sex were found in either experiment. Piglets fed diet 2 in experiment 2 had higher serum cortisol concentrations than the piglets fed the control ration ($P<0.05$; Table 1).

Fasting levels of serum insulin were below 3 mIU/L in all piglets in experiment 2 and in all castrated male piglets in experiment 1. (Figure 2). The three female piglets in experiment 1 had significantly higher fasting insulin concentrations (range 5.0 — 13.0 mIU/L; $P<0.05$). In both experiments the response of insulin concentrations to feeding was statistically significant as expected (Table 1). However, individual variations were very large especially in experiment 2 (Figure 2).

There was a highly significant negative correlation between postprandial serum insulin and cortisol concentrations in experiment 1 ($r = -0.822$; $P<0.01$; Figure 4). This relationship did not occur in experiment 2 ($r = -0.062$; $n = 8$), largely because one piglet, which ate very little, had a high insulin response and high cortisol ($r = -0.485$; $n = 7$). However, postprandial cortisol was negatively correlated with postprandial T4 ($r = -0.762$; $P<0.05$) and preprandial cortisol positively correlated with preprandial insulin in experiment 2 ($r = 0.769$; $P<0.05$).

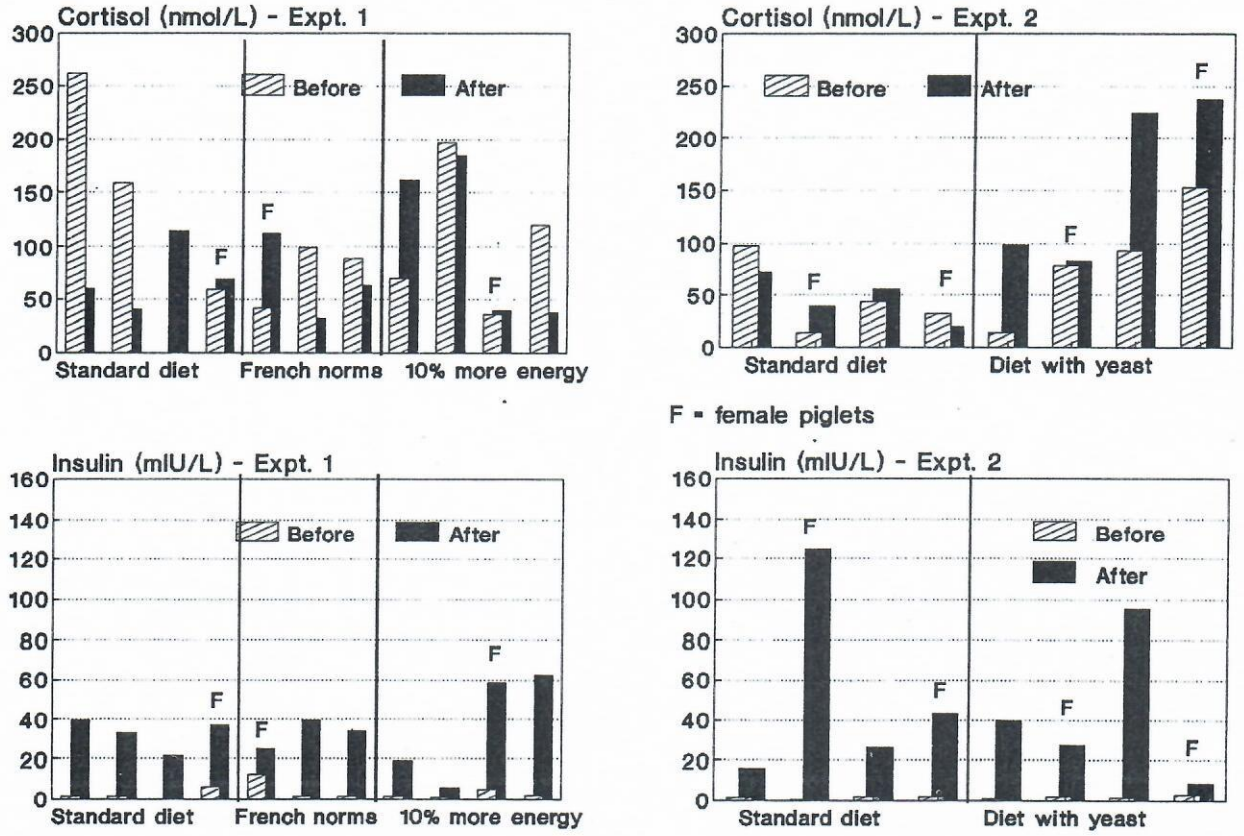

Figure 2. Serum concentrations of cortisol and insulin before and 30 to 45 minutes after feeding weaned piglets (barrows and females) different diets.

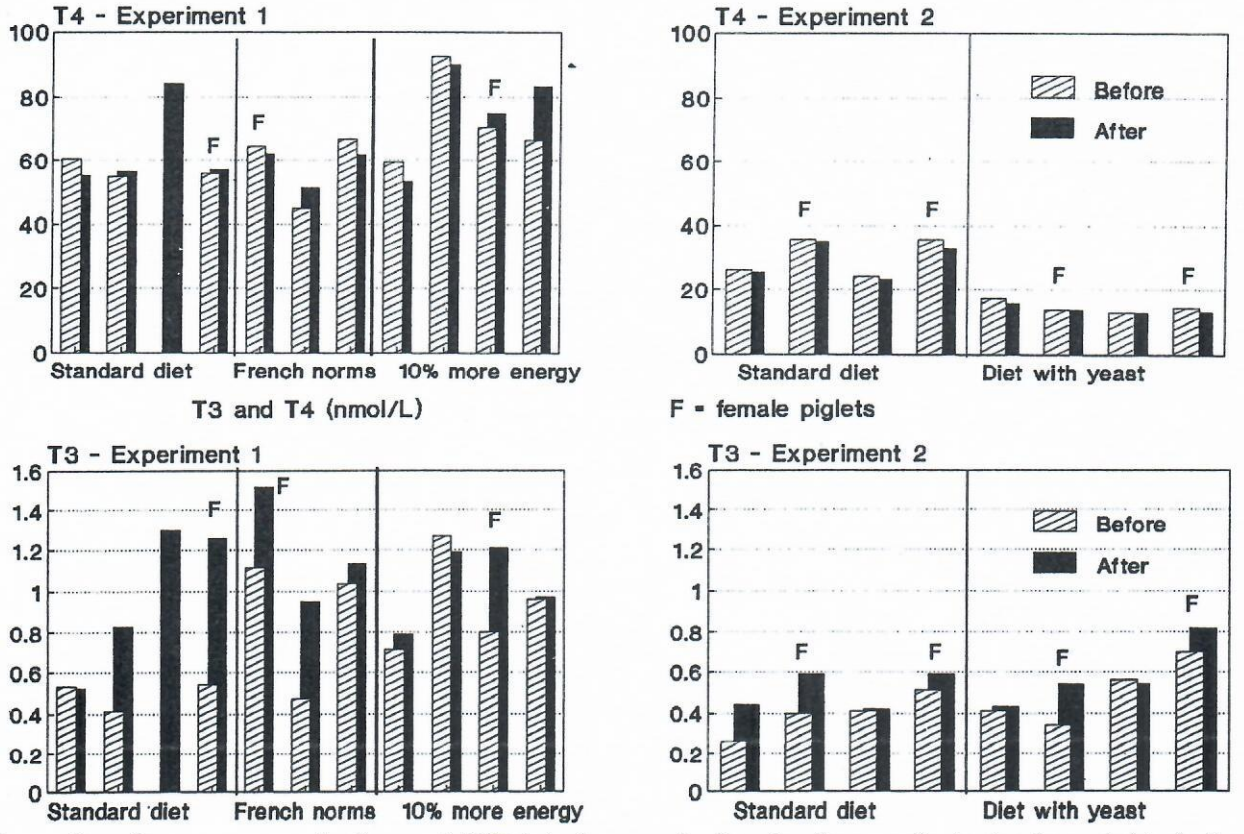

Figure 3. Serum concentrations of IGF-1 before and after feeding, gain in body weight during the experiments and rate of feed consumption between blood collections in weaned piglets (barrows and females) offered different diets.

Serum IGF-1 concentrations tended to be higher in experiment 1 than in experiment 2 (Figure 3). Namely, only one piglet in experiment 1 had levels below 100 arbitrary units per litre, whereas six out of eight piglets had levels between 50 and 100 units per litre in experiment 2. In experiment 1, the female piglets had significantly higher serum IGF-1 concentrations than the barrows ($P<0.01$). Piglets fed diet 2 which contained less crude protein had lower IGF-1 concentrations than piglets fed the other two diets ($P<0.05$; Table 1). Preprandial and postprandial concentrations of IGF-1 were very similar, as expected. In experiment 2 IGF-1 levels were higher in piglets examined on day 2 than on day 1 but the effect was largely due to the value for one animal and no other significant differences were noted in this experiment.

The growth rate of the piglets in the period preceding blood sampling is given in Figure 3. The mean gain in body weight of the piglets in experiment 1 was 435 g per day. A higher rate of gain occurred in experiment 2 (508 g/day; $P<0.05$), although no signiificant differences within the experiments were established for this small number of piglets examined (Table 1). On the basis of the performance of all 36 piglets in experiment 1, Živković and coworkers (1992) concluded that diet 2 gave a slightly lower body weight gain (3.4% less), while diet 3 gave a higher gain (7.7%) and a more favourable feed conversion ratio (7.6%) than diet 1. In experiment 2, diet 2 supported a better growth rate and feed conversion and was more economic than diet 1 (Živković et al., 1993).

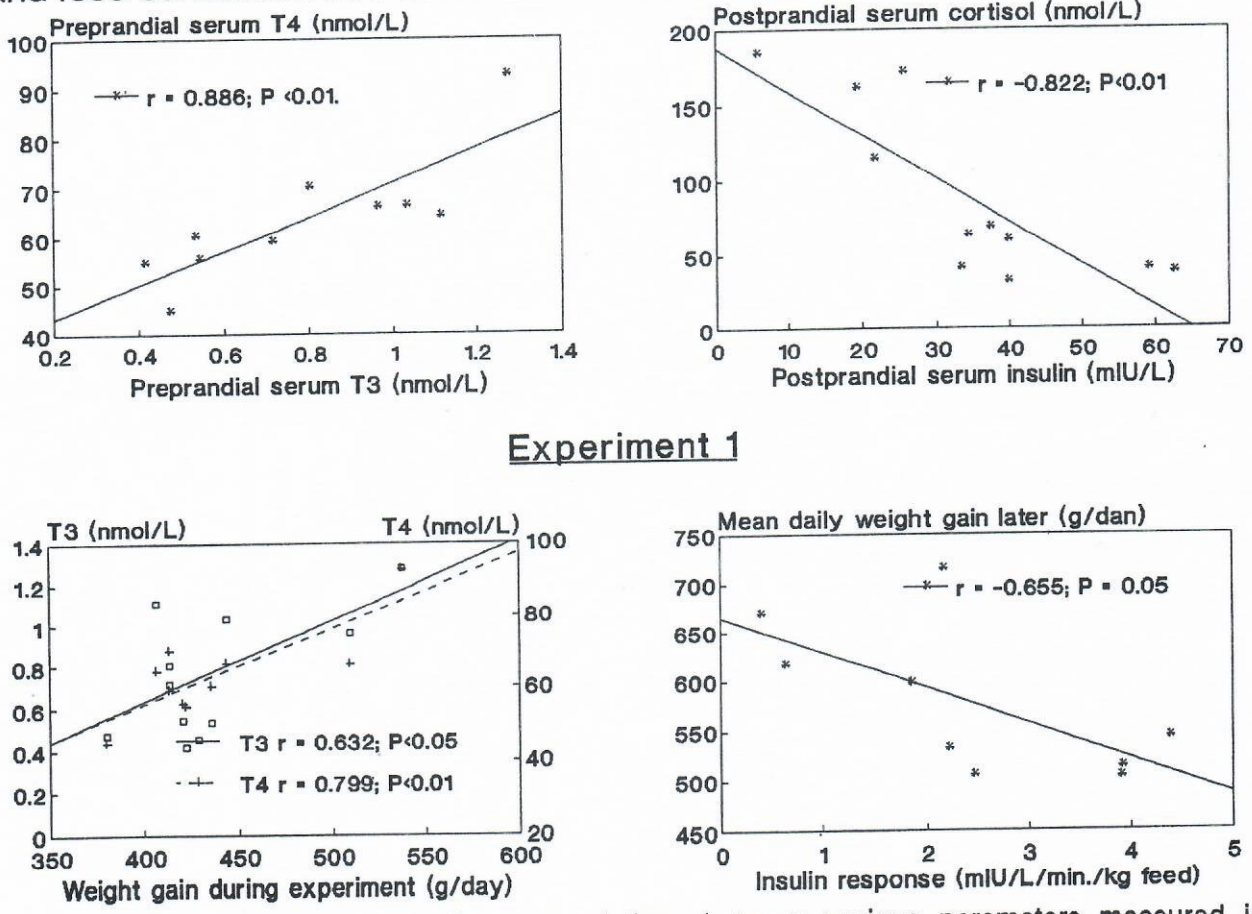

Figure 4. Some statistically significant correlations between various parameters measured in weaned piglets in experiment 1.

There were no statistically significant differences in the amount of feed consumed during the 30 — 45 minute period allowed, between the sexes and the different diets within the experiments (Figure 3) except that the piglets fed the control diet in experiment 2 tended to eat faster than those fed the experimental diet ($P \simeq 0.05$). The piglets ate at a faster rate in Experiment 1 and therefore consumed more in the allotted time.

The rate of growth during the period before blood collection was significantly correlated with preprandial serum concentrations of both T3 and T4 in experiment 1 (Figure 4). Daily gain up to slaughter weight was obtained for eight of these pigs. The only statistically significant relation was a negative correlation with the insulin response per minute per kg feed consumed (Figure 4). In experiment 2 daily gain before blood sampling was positively correlated with preprandial serum T3 ($r = 0.773$; $P<0.05$).

DISCUSSION

The small number of animals examined and the large number of external factors such as season and nutrition would foil any attempt to connect hormone concentrations, performance and the genetic origin of the piglets in the present work. Nevertheless, some interesting conclusions can be drawn from the results obtained.

The large differences between and within the experiments in thyroid hormone levels were unexpected, although the values obtained by other authors vary over a wide range. Thus, De Wilde (1984) found levels of T4 from 40.4 — 91.5 nmol/L and T3 from 0.77 — 1.4 nmol/L in fasted barrows and female pigs of 25 to 100 kg body weight. Dauncey (1990) reported a lower range of concentrations in 10 week old piglets (20 — 42 nmol/L for T4; 0.8 — 1.7 nmol/L for T3), which depended on the energy intake and the environmental temperature at which the piglets were kept. The better performance of the piglets fed the higher energy diet in experiment 1 coincided with higher serum concentrations of thyroid hormones, which confirms their findings. Other authors (Christensen and Just, 1988; Farmer et al., 1991; Yen et al., 1985) found T4 concentrations of 50 — 60 nmol/L and T3 concentrations around 1.0 — 1.5 nmol/L. Thus, the values obtained here in experiment 1 generally fell within the range given by these investigators (overall mean fasting T4 — 62.5 $\pm$ 12.4 and T3 — 0.78 $\pm$ 0.30 nmol/L), whereas the levels found in Experiment 2 were low. The mean concentration for the control diet (29.8 nmol/L) was at the bottom end of the range quoted by the forementioned authors, while that for the experimental diet (14.3 nmol/L) indicated hypothyroidism. There was a minor but significant compensatory increase in T3 (0.45 to 0.54 nmol/L), although T3 levels were below the values given in the available literature. According to the NRC (1988), 0.14 mg iodine/kg diet should satisfy the daily requirement of pigs. Using tabular values (Feodorović — Tome et al., 1970), it was calculated that all three diets in experiment 1 should have contained this amount of iodine, nearly half of which would have been supplied by the fishmeal supplement. In experiment 2 the standard diet contained no wheat and slightly less fish meal.

If the salt supplement had been supplied without added iodine the diet would have been marginally deficient (about 0.11 mgl/kg). whereas diet 2 would have contained only half the amount of iodine required. Nevertheless, no goitres were noticed and the performance of the animals during the experiment was not diminished by the probable deficiency of iodine.

The piglets included in these experiments were subjected to several kinds of stress. First of all they were removed from the feeding group and housed overnight in adjacent individual cages. No feed was available for 16 h and that was followed by the acute stress of two blood collections. Individual piglets reacted to these circumstances in widely different ways as indicated by serum cortisol concentrations. Out of the 19 piglets involved, cortisol levels did not exceed 100 nmol/L in 10 animals for either blood sample. Namely these piglets appeared to be resistant to both nutritional and environmental stress. Three piglets in experiment 1 had high initial cortisol concentrations (>100 nmol/L), which decreased three-fold after feeding, suggesting that liver glycogen stores had probably been exhausted and the high initial cortisol concentrations reflected the requirement for gluconeogenesis. Another piglet in experiment 1 had high cortisol concentrations at both time intervals. This animal also had high T3 and T4 concentrations, a negative response to feeding for T3 levels and a very weak insulin response. Nevertheless it grew well both during the experiment and later.

Four piglets (2 from each experiment) showed marked increases in cortisol concentrations between the two blood collections, indicating that they were susceptible to acute handling stresses (Farmer et al., 1991). According to these authors serum concentrations of cortisol increased from an initial level of about 110 nmol/L to about 275 nmol/L within 15 minutes of initiating a 5 minute acute stress. Values returned to normal within 2 h. The acute responses to this stress of other hormones such as T3, T4 and insulin were over within 30 minutes and therefore would have had no influence on our results if they followed a similar time course in our experiments. Von Borell and Ladewig (1989) concluded that a chronic stress such as tethering can increase the response of piglets (20kg b. wt.) to an acute stressor or ACTH injection. Moreover, individual piglets could be divided into high reacting, middle reacting and low reacting subgroups.

Fasting concentrations of serum insulin tended to be very low in most of the piglets (<3mIU/L), with the exception of the three female piglets in experiment 1. This led to an apparent effect of sex on fasting insulin in this experiment. Since this difference was not found in experiment 2, it may be suggested that the obvious difference in hormone balance between these sets of animals was not actually related to their sex. Moreover, previous results obtained for fasting insulin concentrations in seven female and seven castrated male pigs of 60 — 70 kg body weight showed no effect of sex (mean values – 3.3 $\pm$ 3.5 and 3.5 $\pm$4.6 mIU/L respectively) but there were five animals with fasting insulin concentrations of 4.9 to 11.3 mIU/L (two barrows and three gilts) while the remainder had significantly lower values (0.8 $\pm$ 0.2 mIU/L). This indicated two subpopulations of pigs concerning their reaction to an overnight fast. A similar partitioning unrelated to sex, where low fasting values were encountered in about two thirds

of the piglets was found in subsequent experiments. Other authors recorded fasting concentrations from 4.5 to 10 mIU/L for piglets of various ages and breeds fed standard diets (Atinmo et al., 1976, 1978; Ehrensvärd et al., 1981; Farmer et al., 1991; McCusker and Wangsness, 1979), although levels of 1 — 3 mIU/L were found in piglets given protein deficient rations (3% — 6% CP) by Atinmo and coworkers (1976). Thus fasting insulin concentrations were lower in most of our piglets than those reported in the literature. It is possible that some values given by other authors were obtained with insensitive assays. In our case the first standard was 3 mIU/L, so that values below this level were obtained by extrapolation. Other kits have standard curves starting at 5 mIU/L.

One of the main aims of this investigation was to detect differences in the response of peripheral insulin concentrations to feeding and possible relationships with other hormones and performance. Peak postprandial insulin concentrations occur 30 — 45 minutes after feeding (Christensen and Just, 1988), which is why this interval was chosen here. However, the insulin response was found to be diverse and not related to the other parameters studied.

Due to the lack of a reference standard, the results for IGF-1 were given in arbitrary units. Values within the range 110 —190 μg IGF-1/L were reported by Dubreuil and coworkers (1990) and Farmer and coworkers (1991), whereas Owens and coauthors (1990) found higher concentrations (300 μg/L in young female pigs and 490 μg/L in intact males). Buonomo and coworkers (1987) established that the circulating level of IGF-1 differed considerably between micro and macro breeds of swine at 3 months of age and the concentrations could be increased or decreased by GH treatment or hypophysectomy respectively. However, in our experiment serum concentrations of IGF-1 were not significantly correlated with growth rate or any other parameter measured.
The faster rate of feeding of the piglets in experiment 1 was consistent with the idea that they would have been more hungry in the winter than in the summer. Moreover, the higher levels of thyroid hormones should be associated with a higher basal metabolic rate, greater maintenance requirment and more alert behaviour in experiment 1. McCusker and Wangsness (1979) found that a lean breed of swine (Yorkshire) ate more rapidly (20.1 g feed/minute) than an obese breed (7.1 g feed/minute) but the plasma insulin response per unit of feed consumed was elevated in obese compared to lean pigs. In our investigation the rate of eating was not significantly correlated with any of the other parameters investigated in either experiment.

In conclusion it may be suggested that only serum thyroid hormone levels were associated with the growth rate of the piglets in these preliminary experiments and the association could be related to dietary factors. Namely, in experiment 1 piglets fed the higher energy ration gained faster and had higher T3 and T4 levels. In experiment 2 the greater weight gain in the probably iodine deficent group fed no fishmeal was associated with a slight compensatory increase in T3. The composition of the gain was not determined. As a whole the results indicated the great heterogeneity of the pig population on the investigated farm.

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KONCENTRACIJE NEKIH METABOLIČKIH HORMONA PRE I POSLE HRANJENA I PRIRAST ODBIJENE PRASADI HRANJENIH RAZLIČITIM OBROCIMA

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SADRŽAJ

Određene su koncentracije insulina, kortizola, insulinu-sličnog faktora rasta (IGF-1), tiroksina (T4) i trijodotironina (T3) pre i posle hranjenja odbijene prasadi koja su dobijala različite obroke u dva ogleda zimi odnosno leti.

U ogledu 1, standardni obrok (CP — 20.2%) je bio zamenjen izoenergetskim obrokom koji je sadržao nešto manje sirovih proteina (17. 5%, obrok 2). Obrok 3 je imao sličan sadržaj sirovih proteina (19.6%) obroku 1 ali je sadržao veću koncentraciju energije. Osim vrlo niske koncentracije insulina pre hranjenja kod većeg broja prasadi, nivoi hormona u serumu su bili u opsegu nađenom od strane drugih autora. Nešto bolji prirast prasadi hranjenjem obrokom 3 je bio u značajnoj korelaciji sa povišenim koncentracijama T3 i T4. Prasad hranjena obrokom 2 to jeste sa manje proteina su imala niže koncentracije IGF-1 od ostalih životinja. Koncentracija insulina posle hranjenja je bila u negativnoj korelaciji sa koncentracijom kortizola posle hranjenja, verovatno kao posledica razlika u reagovanju individualnih životinja na prekonočno gladovanje.

U ogledu 2, dodatak ribljeg brašna standardnom obroku je bio zamenjen stočnim kvascem. Iako su prasad, koja su hranjena eksperimentalnim obrokom pokazala povoljniji prirast, nađene su vrlo niske koncentracije tireoidnih hormona u serumu tih životinja, što je ukazalo na deficit joda. Nivoi kortizola i insulina bili su slični onima u prvom ogledu, dok su nivoi IGF-1 bili niži. Zaključeno je da su obrok i godišnja doba verovatno zajednički dozvolili nižu brzinu bazičnog metabolizma kod prasadi u ogledu 2, naročito pri ishrani obrokom 2. Ta prasad su, zatim, bila otpornija na prekonočno gladovanje i konzumirala su obrok sporije ali su porasla brže od prasadi u ogledu 1, jer je količina hranljivih materijala potrebna za održavanje bila manja.