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# THYROID FUNCTION, METABOLIC INDICES AND GROWTH PERFORMANCE IN PIGS FED 00-RAPESEED MEAL

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Thyroid function and morphology, liver morphology, some metabolic indices, haematological parameters and growth performance of fattening pigs fed 00-rapeseed meal (00-RPM) were examined. The control group was fed on a standard diet containing 6% sunflower seed meal (SM) during the growing period and 8% during the finishing period. The first experimental group was fed a diet in which SM was replaced by equal (6% and 8%) amounts of 00-RPM. The second experimental group was fed with a higher (8% and 10%) amount of 00-RPM. There were no significant differences between the control and experimental groups in the serum concentrations of triiodothyronine (T3) and thyroxine (T4). Thyroid gland and liver weights were significantly ($P < 0.01$) higher in both groups fed 00-RPM than in the group fed SM. The epithelium of the thyroid gland was cuboidal or columnar and the follicular area was moderately enlarged in pigs fed 00-RPM. Marked changes in liver histology were not observed. The 00-RPM diet increased ($P < 0.01$) the serum values of total proteins in the first fattening period. At the end of fattening both groups fed 00-RPM had higher ($P < 0.05$; $P < 0.01$) concentrations of plasma glucose than the control group. The inclusion of 10% of 00-RPM during the finishing period increased ($P < 0.05$) the serum values of insulin. Daily weight gain during the growing and the finishing period was higher ($P < 0.05$) in the experimental groups than in the control group. The results suggest that 6–10% 00-RPM can be used as a protein source in the diet of fattening pigs without poisonous side effects.

**Key words:** Pig, rapeseed meal, thyroid hormones, thyroid and liver histology, metabolic indices, haematology, growth performance

Rapeseed meal is considered a valuable protein source for animal feeding because of its well-balanced amino acid composition. However, the presence of antinutritional and toxic factors, particularly glucosinolates, limits the utilisation of rapeseed meal in animal feeding (Bell, 1984; Pusztai, 1989). Breakdown products of glucosinolates slow down the growth of animals and have goitrogenic and hepatotoxic effects (McKinnon and Bowland, 1979; Rundgren, 1983;

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Busato et al., 1991; Bell, 1993; Spiegel et al., 1993*a, b*; Mawson et al., 1994*a, b*). Since pigs are more sensitive to the toxic effects of such compounds, the use of rapeseed meal in pig feeding is less common than in ruminant feeding.

Extensive genetic research for the improvement of rapeseed meal varieties has lately resulted in the cultivation of a rapeseed meal variety that has a very low concentration of glucosinolates called 00-varieties (Bell and Keith, 1991; Farkaš et al., 1993). The low concentration of glucosinolates is indicative of the potential importance of 00-varieties of rapeseed meal in pig feeding. Furthermore, modern processing technologies of rapeseed meal tend to inactivate glucosinolates and reduce hull content in a meal, by which a better digestibility and utilisation of amino acids from the seeds of rapeseed meal in pig feeding is achieved (Bourdon and Aumaitre, 1990; Näsi and Siljander-Rasi, 1991; Valaja et al., 1993; Fauduet et al., 1995; Siljander-Rasi et al., 1996).

On the basis of the results of experiments examined so far, it is not possible to give a definitive recommendation on the amount of presscake produced from 00-varieties that should be added to the meal for pigs with regard to the age and duration of feeding. However, it can be said that negative effects on health are more expressed in pigs up to 20 kg of body weight, and are manifested in considerably lower growth performance. Investigations carried out on fattening pigs during the growing as well as the finishing period of feeding, have not given consistent results. The exception is the influence on the morphology and function of the thyroid gland (Bell and Keith, 1991; Spiegel and Blum, 1993; Spiegel et al., 1993*a*; Lettner et al., 1996; Moreira et al., 1996; Schöne et al., 1996). Investigations are especially lacking as to the effect of feeding rapeseed meal on several metabolic processes.

In this work we investigated thyroid hormones, some metabolic indices and haematological parameters in the blood of fattening pigs fed increasing amounts of 00-RPM. Thyroid gland and liver histology was also studied. In addition, studies on growth and feed utilisation were performed.

## Materials and methods

### *Animals, housing and feeding*

Investigations were carried out on 90 healthy pigs (crossbreds of Landrace, Large White and Pietrain) bred on the farm in which these experiments were carried out. After weaning at 23 days of age, when the pigs weighed approximately 6.5 kg, they were fed a starter diet till the age of 55 days and a mean body weight of 16 kg. The starter diet contained 19.5% proteins of which 6.2% were from soybean. From 16 kg of body weight the pigs were fed a grower diet containing 18% proteins of which 8.7% were from soybean. When the pigs reached approximately 22 kg of body weight, at the mean age of 75 days, they were divided by the method

of random choice into three feeding groups, each consisting of 30 animals (15 castrated males and 15 non-castrated females). The first (control) group was fed a standard diet which contained 6% of SM during the growing period and 8% during the finishing period. The second group was fed a diet in which sunflower seed meal had been replaced by the equivalent amount (6% and 8%) of 00-RPM. The third group was fed an increased amount of rapeseed meal (8% in the first and 10% in the second fattening period; Table 1). The level of glucosinolates in rapeseed presscake meal amounted to 30.13 μM/g of dry matter.

**Table 1**

Survey of feeding groups of pigs according to fattening period

| | Fattening periods* | Feeding groups | | |
|---|---|---|---|---|
| | | SM (Control) | 00-RPM I (Experimental) | 00-RPM II (Experimental) |
| 1 | 0 to 42nd day | 6% | 6% | 8% |
| | | Each group received 16% proteins in meal | | |
| 2 | 43rd to 112th day | 8% | 8% | 10% |
| | | Each group received 14% proteins in meal | | |

Abbreviations: SM = sunflower seed meal; 00-RPM = 00-rapeseed meal. *0 day indicates the start of experimental feeding when average age of the pigs was 75 days (minimal age 69 days and maximal 76 days) and body weight was approximately 22 kg

Pigs were held in a house made up of 44 compartments. There were three pigs in every compartment. Each compartment had one half of a grate-like floor, and its dimensions were 1.2 × 4 m. This means that the floor area was 0.96 $m^2$ per animal. Every compartment had a feeder with a basket for feeding *ad libitum*. All pigs had free access to water from an automated watering-trough.

*Blood collection and analyses*

Blood samples for biochemical and haematological analyses were taken from the v. cava cranialis twice: at the end of the growing (42nd day of experiment) and of the finishing (112th day of experiment) period of feeding. The concentrations of T3 and T4 in the serum were determined by an immunochemical method using the test of DELFIA Wallac (Finland). The concentration of insulin in serum samples was determined by the RIA method of Kabi Pharmacia Diagnostics (Sweden). Blood glucose and serum cholesterol concentrations were measured by an enzymatic method using the reagents and test of the company TRACE (Australia). Total proteins in the serum were determined by a photometric method based on the biuret reaction.

Erythrocyte count, haematocrit value and haemoglobin concentration were determined by using an automated multi-channeled blood counting system (Coulter Counter, model ZF). A supravital staining of blood smears with methylene blue was performed to determine the proportion of reticulocytes within the number of erythrocytes.

*Histological analyses of the thyroid gland and liver*

After the pigs had been slaughtered at the slaughterhouse, the livers and the thyroid glands were excised and weighed immediately. The samples of both organs of each animal were fixed in formalin. After fixation for 24 h the samples were embedded into paraffin wax, then sections were cut with a microtome and stained with haematoxylin and eosin.

*Statistical analysis*

The means and standard errors were calculated for each group. The differences between mean values of examined parameters of pigs were accepted as significant when the error probability was less than 5% ($P < 0.05$).

## Results

*Growth performance and feed utilisation*

Table 2 shows that pigs fed 00-RPM, as an equivalent or an increased substitute for SM, had significantly ($P < 0.05$) higher body weight gain in the first fattening period. During the second fattening period the body weight gain of pigs fed 00-RPM as equivalent substitute for SM was higher ($P < 0.05$) again. The conversion of feed into body weight during the first fattening period was somewhat better in both groups fed 00-RPM, but was not statistically different from the control.

*Thyroid gland and liver weights*

The average weight of thyroid glands in the control groups of pigs was 9.396 g (Table 3). In groups fed 00-RPM the thyroid glands weighed in average 13.764 g and 15.021 g, respectively. In other words, the weight of the thyroid glands increased by about 46% ($P < 0.01$) and 59% ($P < 0.01$), respectively.

Pigs fed SM had an average liver weight of 1.401 kg. In pigs fed 00-RPM the average liver weight amounted to 1.807 kg and 1.773 kg, respectively (Table 3). This means that the liver weight of these pigs had increased by about 29% ($P < 0.01$) and 26% ($P < 0.01$), respectively.

**Table 2**

Growth performance and feed utilisation in pigs fed sunflower seed meal (SM) or 00-rapeseed meal (00-RPM)

| Parameters | Fattening period | | | | | |
|---|---|---|---|---|---|---|
| | 0–42nd day | | | 43rd–112th day | | |
| | SM | 00-RPM | | SM | 00-RPM | |
| | 6% | 6% | 8% | 8% | 8% | 10% |
| Pigs in group (n) | 30 | 30 | 30 | 29 | 30 | 28 |
| Initial weight (kg)$^{+}$ | 22.94 | 22.10 | 21.35 | 55.10 | 56.76 | 55.76 |
| Feeding days (d) | 42 | 42 | 42 | 70 | 70 | 70 |
| Average weight gain: (kg/pig) | 32.183 | 34.667$^{*}$ | 34.416$^{*}$ | 51.339 | 53.536$^{*}$ | 51.276 |
| (kg/d) | 0.766 | 0.825$^{*}$ | 0.819$^{*}$ | 0.745 | 0.787$^{*}$ | 0.765 |
| Feed/ gain: (kg/kg) | 2.769 | 2.644 | 2.615 | 3.539 | 3.574 | 3.527 |
| (kg/d/pig) | 2.142 | 2.182 | 2.143 | 2.677 | 2.814 | 2.707 |

Abbreviations: SM = sunflower seed meal; 00-RPM = 00-rapeseed meal; $^{*}$Significantly different ($P < 0.05$) from control (SM feeding group). $^{+}$Initial weight is the average weight of pigs within groups at the start (0 day) of experimental feeding

**Table 3**

Mean values of body weight and the weights of thyroid glands and liver of fattening pigs fed sunflower seed meal (SM) or 00-rapeseed meal (00-RPM)

| Feeding groups | Fattening period | | Feeding (d) | n | Body weight (kg) | Thyroid weight (g) | Liver weight (kg) |
|---|---|---|---|---|---|---|---|
| | 0–42nd day | 43rd–112th day | | | | | |
| SM | 6% | 8% | 112 | 29 | 106.439 | 9.396 | 1.401 |
| 00-RPM | 6% | 8% | 112 | 30 | 110.296 | 13.764$^{*}$ | 1.807$^{*}$ |
| 00-RPM | 8% | 10% | 112 | 28 | 107.036 | 15.021$^{*}$ | 1.773$^{*}$ |

Abbreviations: SM = sunflower seed meal; 00-RPM = 00-rapeseed meal. $^{*}$Significantly different ($P < 0.01$) from the respective control (SM feeding group)

### *Histopathology of thyroid gland and liver*

In the group fed 6% and 8% of 00-RPM, a mild to moderate (Fig. 1) hypertrophy of the follicular epithelium was found in 12 animals, whereas six animals had a severe hypertrophy of the epithelium. Pigs fed an increased amount of 00-RPM (8% and 10%) also had a mild hypertrophy of the follicular epithelium in the case of four animals, a medium hypertrophy in 12 animals, and both hypertrophy and hyperplasia of the epithelium in four animals (Fig. 2).

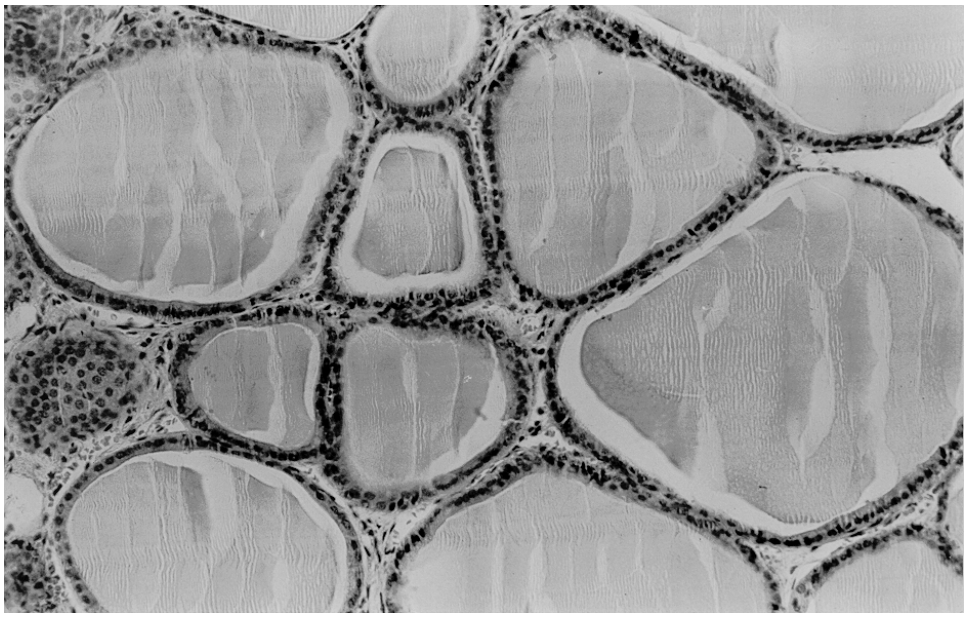

*Fig. 1.* Moderate hypertrophy of the follicular epithelium of the thyroid gland of a pig fed 6% and 8% of 00-RPM. Haematoxylin and eosin (HE) staining, magnification × 50

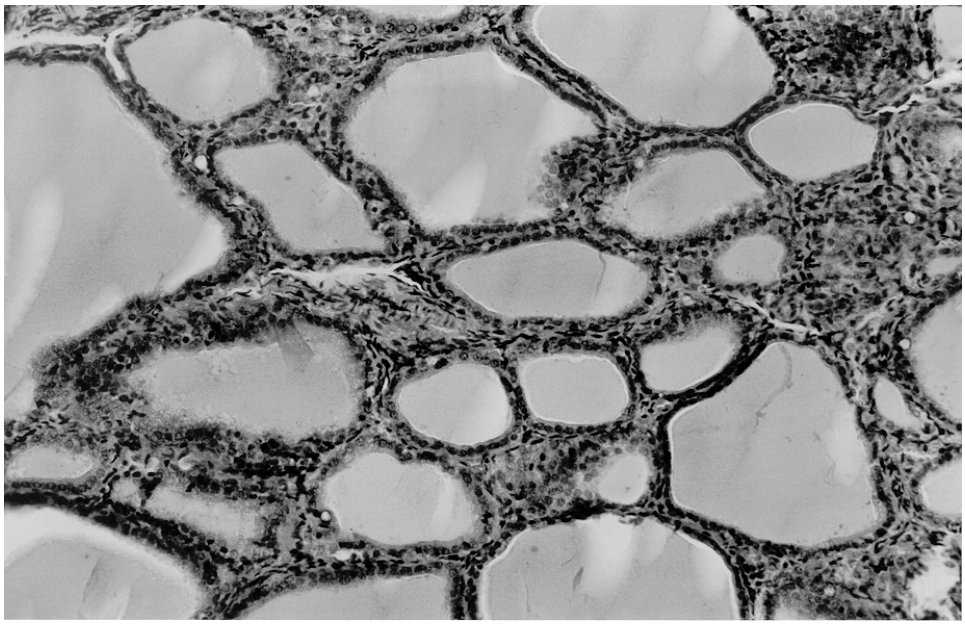

*Fig. 2.* Hypertrophy and hyperplasia of the follicular epithelium of the thyroid gland of a pig fed 8% and 10% of 00-RPM. Haematoxylin and eosin (HE) staining, magnification × 50

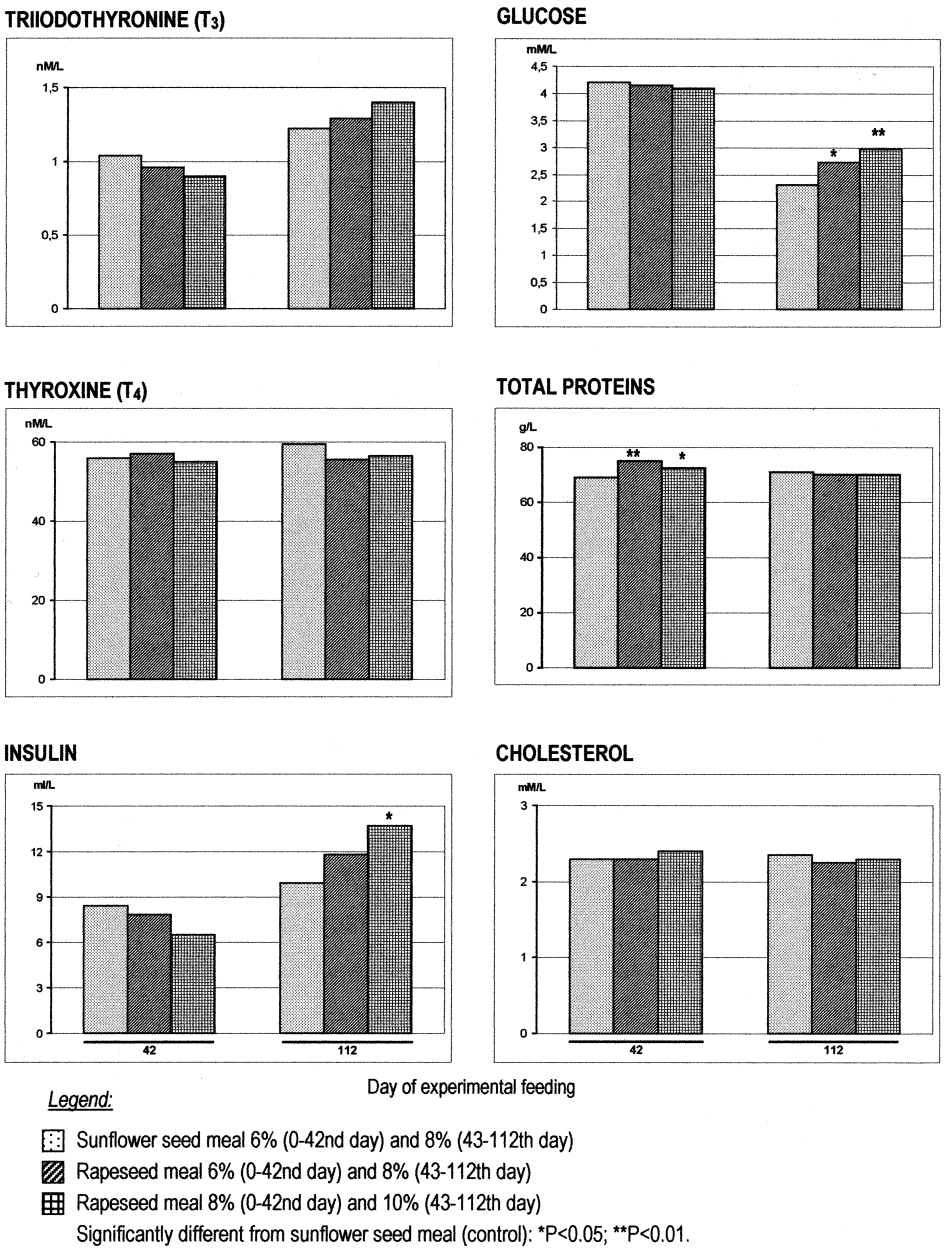


*Fig. 3.* Mean values of hormone and metabolite levels in the serum of pigs fed sunflower seed meal or rapeseed meal

**Table 4**

Mean values of biochemical and haematological parameters in the blood of pigs at the end of the growing (42nd day) and finishing period (112th day) of fattening

| | Fattening period | | | | | |
|---|---|---|---|---|---|---|
| | 1 (0–42nd day) | | | 2 (43rd–112th day) | | |
| | SM | 00-RPM | | SM | 00-RPM | |
| | 6% | 6% | 8% | 8% | 8% | 10% |
| Feeding days | 42 | 42 | 42 | 70 | 70 | 70 |
| Pigs in group | 30 | 30 | 30 | 29 | 30 | 28 |
| Biochemical parameters[+] | | | | | | |
| Triiodothyronine (nM/L) | 1.05 (0.08) | 0.96 (0.06) | 0.91 (0.04) | 1.22 (0.06) | 1.28 (0.05) | 1.39 (0.08) |
| Thyroxine (nM/L) | 55.18 (3.99) | 57.73 (2.74) | 54.94 (2.60) | 59.10 (2.53) | 56.00 (1.91) | 57.58 (2.74) |
| Insulin (mI/L) | 8.50 (1.50) | 7.88 (0.95) | 6.44 (0.57) | 10.05 (0.90) | 11.86 (1.24) | 13.6* (1.27) |
| Glucose (mM/L) | 4.26 (0.15) | 4.20 (0.18) | 4.10 (0.14) | 2.32 (0.13) | 2.73* (0.15) | 2.96** (0.14) |
| Total proteins (g/L) | 69.45 (1.10) | 74.95** (1.39) | 72.18* (1.06) | 71.91 (1.24) | 71.22 (1.17) | 71.22 (1.07) |
| Cholesterol (mM/L) | 2.37 (0.05) | 2.37 (0.05) | 2.47 (0.06) | 2.39 (0.08) | 2.28 (0.07) | 2.29 (0.06) |
| Haematological parameters* | | | | | | |
| Erythrocytes ($\times 10^{12}$/L) | 6.39 (0.33) | 6.16 (0.19) | 6.24 (0.13) | 6.96 (0.23) | 6.61 (0.44) | 6.78 (0.39) |
| Reticulocytes (%) | 1.64 (0.26) | 1.71 (0.17) | 1.68 (0.18) | 1.43 (0.09) | 1.20 (0.10) | 1.49 (0.14) |
| Haemoglobin (g/L) | 124.80 (9.08) | 114.38 (3.98) | 116.22 (2.63) | 130.20 (7.44) | 120.50 (9.31) | 126.36 (6.27) |
| Haematocrit (%) | 44.00 (3.68) | 40.65 (1.25) | 42.09 (0.82) | 44.88 (2.84) | 46.67 (3.41) | 47.34 (2.71) |

Abbreviations: SM = sunflower seed meal; 00-RPM = rapeseed meal. [+]Biochemical parameters were determined in 25–29 and haematological parameters in 20–28 blood samples of pigs from each group. Significantly different from control (SM feeding group): $^{*}P < 0.05$; $^{**}P < 0.01$. SEM in parentheses

Histopathological changes of the liver parenchyma were present only in some cases, and were manifested as a lipid infiltration and parenchymal dystrophy of hepatocytes. In the interstitium of the liver a passive hyperaemia of various degrees was found in pigs fed SM and 00-RPM.

*Blood biochemical constituents and haematology*

The concentration of serum T3 in both groups of pigs fed 00-RPM was lower at the end of the first fattening period (42nd day), and higher at the end of fattening (112th day) than in pigs fed SM (Fig. 3 and Table 4). However, these differences were not confirmed as statistically significant. It is interesting that by the end of the fattening period serum concentrations of T4 were lower in the groups fed 00-RPM than in the control group, which is opposite with the above-mentioned changes in T3 concentration.

Blood glucose concentration did not differ among groups at the end of the first fattening period. However, at the end of the fattening, the serum glucose concentration of the two groups fed 00-RPM was significantly higher ($P < 0.05$; $P < 0.01$) than that of the group fed SM. At the end of the second fattening period, insulin was significantly higher ($P < 0.05$) in the serum of the group fed an increased amount of 00-RPM when compared to the group fed SM.

At the end of the first fattening period, serum total protein concentration was significantly higher in both experimental groups ($P < 0.01$; $P < 0.05$) than in the control group. These differences, however, no longer existed at the end of the second period. Moreover, the concentrations of total proteins were then almost identical in the sera of all feeding groups.

The serum concentration of cholesterol measured at the end of the first and second fattening period did not differ among groups. Also, there were no significant changes in serum cholesterol concentration according to the age of the animals.

There were no significant differences between groups in any of the erythrocyte parameters. Haemoglobin concentration increased in pigs of all groups during the second fattening period. The proportion of immature erythrocytes (reticulocytes) among erythrocytes was constant throughout the experiment.

## Discussion

*Thyroid morphology and function*

Our observations about the health of pigs fed 00-RPM were primarily based on morphological and functional characteristics of the thyroid gland. Concerning the size of the thyroid gland, which was estimated according to the absolute weight of the gland, we have found that hypertrophy of the thyroid gland increases with the amount of RPM in a diet, which is in agreement with the results of many authors (McKinnon and Bowland, 1979; Bell, 1984; Näsi et al., 1985; Busato et al., 1991; Spiegel et al., 1993*a, b*). Namely, our pigs which received 6% to 10% of RPM, produced from 00-varieties which contain only 30.13 μM of glucosinolates per g, had a thyroid gland weight increased by about 46% and 59%, respectively. This hypertrophy is relatively slight compared with

the strong goitrogenic effect of RPM produced from rapeseed varieties that have a high concentration of glucosinolates. However, it has also been shown that 00-RPM can influence the size and function of the thyroid gland in pigs even when the amount of glucosinolates per gram of dry matter is not higher than 5 μM, if the share of such presscake in a meal is more than 15% (Pusztai, 1989; Stoll, 1996).

In the group of pigs that received 6% and 8% of 00-RPM, most animals had developed a mild, moderate or severe hypertrophy of the follicular epithelium. Pigs that were fed an increased amount of 00-RPM (8% and 10%) developed almost the same changes of follicular epithelium as did the pigs from the group previously mentioned. However, in a few pigs, along with a strong hypertrophy, hyperplasia of the epithelial cells was also found, which was probably induced by a somewhat higher amount of the glucosinolates. The presence of columnar cells, and especially the hyperplasia of the follicular epithelium, is the result of increased stimulation of the thyroid gland by thyroid-stimulating hormone (TSH). However, there was no case of degenerative histological changes that could be ascribed to the hypofunction or the collapse of the thyroid gland. Serum concentrations of T3 and T4 in both groups of pigs which were fed 00-RPM did not differ statistically at the end of the first and second feeding period from the concentrations measured at the same time in sera of the group fed SM. However, it is of interest to note that the serum level of T3 was lower at the end of the growing period, and higher at the end of the finishing period in groups fed 00-RPM than in the control group. Since T3 has four times stronger biological effects than T4, it is considered that an increased conversion of T4 into T3 during the decreased secretion of T4, automatically compensates the biological consequences of the decreased amount of T4 in the organism (Danforth and Burger, 1989; Felig et al., 1995).

*Liver morphology*

Pigs fed 00-RPM had an enlarged liver; however, differently from the thyroid gland, the increase of the amount of 00-RPM in a meal (from 6% to 8% in the first and from 8% to 10% in the second fattening period) did not cause any further enlargement of the liver. Also, the histological abnormalities of the liver parenchyma were not found to increase as the amount of 00-RPM in the meal was increased. It is not clear whether liver hypertrophy is caused only by glucosinolates or by some other constituents of rapeseed meal as well. According to the results of Spiegel et al. (1993*a, b*), rapeseed meal feeding decreases the activity of liver T4,5-deiodinase, an enzyme which splits T4 (Danforth and Burger, 1989). Therefore the liver enlargement caused by RPM feeding can be considered as a compensatory mechanism by which the liver tends to compensate for the decreased activity of T4,5'-deiodinase, similar to how the hypertrophy of the thyroid gland compensates for the decreased synthesis of their hormones. It is assumed that, besides glucosinolates, goitrin is one of the most important sub-

stances responsible for the above-mentioned effect of rapeseed meal on the liver (Langer et al., 1984).

In a few cases the enlarged livers showed histopathological changes such as lipid infiltration. Since these changes also appeared in a few cases among pigs of the control group, we suppose that lipid infiltration is most probably caused by intensive feeding.

*Metabolic indices, haematology and growth performance*

The amount of proteins in the blood was significantly higher at the end of the growing period in both groups fed 00-RPM meal than in the control group. Since those groups had a higher daily weight gain, we presume that a more abundant synthesis of muscle proteins is the result of a well-balanced amino acid composition of rapeseed meal. At the end of the finishing period, pigs fed 00-RPM had a significantly higher concentration of glucose and insulin in the blood than pigs fed SM. Spiegel et al. (1993*a*) found lower concentrations of glucose in the blood of pigs fed 15% 0-RPM, but not in pigs fed 15% 00-RPM, and ascribed this to the lesser consumption of tasteless food, which also resulted in a decreased weight gain. We suppose that the increased level of glucose and insulin in our experimental pigs is, at least partly, a result of the well-balanced meal composition with regard to energy values, amino acids and a high content of essential fatty acids in rapeseed meal.

We have not found significant differences in the serum concentration of cholesterol between pigs fed SM or 00-RPM. However, cholesterol level decreased during the finishing period in pigs that received 00-RPM, which may be associated with the high amounts of essential and polyunsaturated fatty acids in rapeseed meal (Migdal, 1991; Morgan et al., 1992; Nürnberg et al., 1994; Jørgensen et al., 1996). Generally, it is well known that the concentration of cholesterol in the serum is inversely proportional to the activity level of the thyroid gland. Moreover, there is a good correlation between the degree of hypothyroidism and cholesterol level in the blood of fattening pigs (Forenbacher et al., 1968; Zdelar and Forenbacher, 1987).

Feeding 00-RPM in our experiments did not have any negative effect on haematopoiesis. Erythrocyte count, haematocrit and haemoglobin concentrations were similar in all groups. The proportion of reticulocytes within the number of erythrocytes indicates good blood regeneration in all feeding groups of pigs throughout the experiment.

It can be stated that feeding 00-RPM did not have any negative effect on animal growth. Moreover, body weight gains proved to be better in the experimental groups than in the control, especially when SM was replaced by an equivalent (6%) amount of 00-RPM. Moreira et al. (1996) have also shown that fattening pigs receiving 6% of 00-RPM gained more weight than those from the control group fed an isoenergetic diet based on corn and soybean. According to

the results of Jost and Bracher-Jakob (1992), pigs after the age of five weeks can be fed 5–10% 00-RPM without any harmful effects on health and feed conversion.

In conclusion, hypertrophic and hyperplastic changes of the follicular epithelium accompanied by normal concentrations of thyroid hormones in the blood of pigs fed 6–10% 00-RPM suggest that these pigs can retain normal function of the thyroid gland. No pathological changes occurred in other blood biochemical constituents during 00-RPM feeding. Furthermore, the presence of 6–10% 00-RPM in the diet of growing-finishing pigs resulted in effective body weight gain and efficient feed conversion to body mass. Therefore, the results suggest that 00-RPM in amounts up to 10% can be used as a protein source in the diet of fattening pigs without the risk of toxic side effects.

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