

The performance of young pigs fed different amounts of marigold (*Calendula officinalis*) meal; a pilot study

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Abstract

Hexane-extracted calendula meal was tested in an acceptance trial with eighty 8–13 weeks old crossbred pigs to determine their response to diets containing 0, 2, 6, 10 or 20% calendula meal. Performance parameters included feed intake, daily growth and *post mortem* histopathological examination of vital organs. Pigs fed a diet containing 2% calendula meal consumed significantly more feed than the ones fed a calendula-free diet. As the calendula meal content of the diet increased, feed intake tended to decrease.

Post mortem examination of vital organs showed statistically significant ($P < 0.10$) between-treatment differences in heart, kidney, thyroid and pancreas size expressed as percentage of bodyweight.

The results of blood and blood serum analyses for haematocrit, haemoglobin, oxygenated haemoglobin, aspartate-aminotransferase, alanine-aminotransferase, lactate dehydrogenase, creatinine and zinc remained within the reference values for young pigs.

Although calendula meal showed potential as a ration ingredient for young pigs it is advised not to include more than 10% of it in the diet.

Additional keywords: by-products, feed intake, bodyweight gain, blood parameters, *post mortem* examination

Introduction

In recent years, interest has been shown for various industrial applications of oil and specific fatty acids, such as calendic acid, extracted from marigold (*Calendula officinalis* L.) seeds (Steg *et al.*, 1994). Attempts were made to utilize calendic acid in paints, resins and foams, while by-products from the various processing techniques were evaluated as a potential animal feed ingredient.

After seed processing for oil recovery, large amounts of seed mass remain. Economic application of this residue can be decisive for the perspectives of marigold as an agricultural crop in Europe. Traditionally, various oilseed by-products, such as

rapeseed, sunflower and soya bean meal, have been successfully applied as livestock feed ingredients (Hof, 2000). Utilization of calendula meal (CM) as a livestock feed would almost certainly add to the economic viability of the crop and encourage its acceptance in agriculture and industry. The present study was undertaken to provide additional information on chemical characteristics and nutrient availability of any by-products that become available during the extraction of oil from calendula seeds.

An animal acceptance trial was performed to determine whether any detrimental effects would arise from feeding calendula meal. Literature (Hindle & Cone, 1999) indicates that the marigold plant can contain 'traces' of the following substances: alcohol extract (found in the flowers), omega-6-fatty acids (seeds), faradiol mono-ester (flowers), usradiol (flowers), helianol (leaves, stems and roots), 3-O-beta-D-glucuronide (leaves), antheraxanthin (flowers), lutein epoxide (flowers) and triterpene glucosides (leaves, stems, roots and flowers). Although many investigations have been reported, there is little available information on seed extracts and their possible toxicity to animals and human beings. Medicinal properties have been claimed for various extracts from the marigold plant, but no study is known that investigated the possible toxic effects. For example, when this trial started no information was available on the detrimental effects of saponins or their cyanogenic derivatives that, theoretically, could be present in calendula seed by-products.

In the acceptance trial young pigs were fed diets containing varying amounts of CM to monitor feed intake, changes in body weight and blood parameters. Additionally, a *post mortem* examination was performed on vital organs (heart, kidneys, liver, pancreas and thyroid) of several animals. Young pigs were chosen for the experiment rather than other animal species because piglets are considered to have a very sensitive digestive system and react instantly to any changes in their diet.

Materials and methods

Preparation of calendula meal

CEBECO (Rotterdam, The Netherlands) developed a promising processing technique designed by TNO-MEP (Apeldoorn, The Netherlands) to improve efficiency of separation of hulls from seed. It implies a two-step cleaning process that starts with the removal of the seed hulls from the uncleaned harvest material in a regular hammer mill (Peppink AN 300, Ochten, The Netherlands; specifications: 6-head hammer cross; 3000 rpm; feed stream 250 kg h⁻¹) followed by the separation of hulls and seeds into a fibre stream, an oilseed stream, dust and coarse particles (stones etc.). The system separates particles on the basis of density and shape (aerodynamic resistance; Janssens, 2000).

Hull removal and separation of marigold seeds results in the fractions: hulls, whole seeds and kernels, kernels, small stones and other dense objects (including weed seeds and dust). Hull removal improves oil purity and increases the percentage of oil recovered from the seed. It furthermore leads to potentially advantageous changes in feed quality, due to a lowering of the lignin levels in samples of meal pro-

duced from the extracted seed. In this study, samples from calendula seeds from which the hull had been removed using the previously mentioned method, contained on average 17% crude protein in the dry matter (DM). After extraction with hexane this was increased to 22%. The seed meal examined in this study contained 249 g protein and 143 g lignin per kg DM, with an organic matter digestibility *in vitro* of 49% for ruminants (Tilley & Terry, 1963) and 40% for pigs (Van Der Meer & Perez, 1992). The processed meal used in this experiment originated from the kernel fraction.

Feed preparation

Details on the composition of the ration ingredients are given in Table 1. Each diet contained a basal mixture of 0, 2, 6, 10 or 20% CM supplemented with varying amounts of soya bean meal, soya bean hulls, wheat bran and sunflower seed meal. This basal mixture was added to a mixture of a standard starter pellet (concentrate mixture) from a local manufacturer (Arkervaat-Twente, Nijkerk, The Netherlands). Due to contractual agreements made with growers in England and the Netherlands at the onset of this project, there was only sufficient CM available for the production of one-tonne batches of each diet. This limited the length of the animal experiment.

Experimental design

The acceptance trial was set up with 80 piglets (42 castrates and 38 females) from the cross Dutch landrace x Large White. The animals were randomly assigned to 10 groups of 8 piglets housed in 10 pens distributed over two identical rooms. Groups were fed separately and daily feed intake for each group was calculated as the difference between the amount of feed offered and the amount of feed remaining prior to feeding the next morning. Water was available *ad libitum* via a drinking nipple in each pen. The experiment lasted for 37 days.

The trial comprised 5 treatments in duplicate. A treatment was defined as the amount of CM used to substitute sunflower seed meal, wheat bran, soya bean meal and hulls in varying amounts (see Table 1). Diets contained 0, 2, 6, 10 or 20% CM on the basis of supplementation of the crude protein content. Linear programming was used to balance each ration on the basis of standard requirements for protein, energy, vitamins and minerals (Anon., 1998a).

At the beginning of the experiment the animals weighed, on average, approximately 18 kg. Each animal was weighed on day 1, day 17, day 30 and day 37 of the experiment. Blood samples were taken from the *vena clavicula* (shoulder) of each animal on day 1 and day 37 to provide information concerning blood composition and general health status of the animals.

After completion of the trial all animals fed the control (0% CM), the 10% or 20% CM diets were euthanized by intravenous administration of sodium pentobarbital (Nembutal[®], Sanofi, Nijmegen, The Netherlands) followed by exsanguination via the auxiliary artery. A thorough gross pathological examination was performed and the heart, liver, both kidneys, the thyroid gland and pancreas were extracted from the

Table 1. Feed composition (g kg⁻¹) of the diets used in the feeding trial.

Component ¹	% calendula meal in diet				
	0	2	6	10	20
Pre-mix	7.3	7.3	7.3	7.3	7.3
Concentrate mixture	699.8	699.8	699.8	699.8	699.8
Soya bean meal (CF 50–70, CP > 440)	102.9	101.9	99.9	97.9	92.9
Soya bean hulls (CF > 310)	40.0	36.0	28.0	20.0	0
Wheat bran	90.0	81.0	63.0	45.0	0
Sunflower seed meal (CF > 240)	60.0	54.0	42.0	30.0	0
Calendula meal	0	20.0	60.0	100.0	200.0
Total mix	1000	1000	1000	1000	1000

¹ CF = crude fibre (g per kg DM); CP = crude protein (g per kg DM).

body and weighed after the removal of fat and other connective tissue from the organs. Tissue samples for histological examination were taken from liver, kidneys and thyroid glands. These samples were fixed in a 4% buffered formaldehyde solution and representative samples were embedded in paraplast. Tissue sections were cut and stained with hematoxylin-eosin for histo-pathological examination.

Chemical analysis

The calendula meal and the diets were analysed for contents of dry matter, ash, nitrogen (N; crude protein = N x 6.25), ether extracts (EE), crude fibre (CF) neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and gross energy value (GE) (Steg *et al.*, 1990; Van Vuuren *et al.*, 1991). Amino acid composition was also determined (Van Vuuren *et al.*, 1992).

Blood samples from each animal were analysed for haematocrit, haemoglobin and oxygenated haemoglobin levels. Blood serum was examined for concentrations of aspartate-aminotransferase, alanine-aminotransferase, lactate dehydrogenase, creatinine and zinc. The results were used primarily as an indication of the health status of the animals.

Data analysis

An analysis of variance (ANOVA; Anon., 1998b) was performed on the results to determine statistical significance of treatment effects on bodyweight, bodyweight increase during the intervals between weighing, and bodyweight changes over the whole trial period. ANOVA was also performed on the weights recorded for the specific organs after autopsy.

Results and discussion

Chemical composition of the diets

Results of the chemical analyses of the diets (Table 2) show that ash content (or organic matter) did not differ between diets. Crude protein (CP) levels averaged 21.4% and were comparable between diets. The CP content of the CM was 27.8%, which is much higher than the level (19%) found by Steg *et al.* (1994). The diets did not differ in fat (EE) content (about 6%) except for the 20% CM diet, which contained slightly less EE (5.5%), yet differed little in fibrous components (NDF, ADF and ADL). The percentage CM did not affect the gross energy (GE) levels in the diets.

Another difference with earlier CM samples was an almost 10% lower non-amino acid nitrogen content, but this remains 5% above non-amino acid nitrogen levels measured in rapeseed meal. In the control diet (0% CM) the CP consisted for almost 100% of amino acids but as the percentage CM in the diets increased, CP decreased by up to 5% (Table 2). The CP of the CM used in the basal mixture consisted for only 77% of amino acids.

Amino acid content

The amino acid composition of the CM used in our study (Table 3) differed from the one reported earlier by Steg *et al.* (1994). Higher levels were found for all amino acids determined (Figure 1). Most striking were the high levels of the essential amino acids methionine, cystine and lysine, possibly as a result of seed hull removal. In spite of being higher, amino acid levels, including methionine, cystine and proline, remained below those expected in a more conventional feed such as rapeseed meal (Anon., 2000).

Figure 1 presents a comparison of the amino acid contents that differed most between CM and the substituted ingredients (soya bean meal, sunflower seed meal and rapeseed meal) based on the latest data available in the Dutch Feed Table (Anon.,

Table 2. Chemical composition and gross energy content of the calendula meal and the calendula-meal containing diets fed to young pigs.

CM ¹ (%)	DM ² (g)	Ash	CP	AA	CF	EE	NDF	ADF	ADL	GE (MJ per kg DM)
----- (g per kg DM) -----										
0	881	67	208	207	53	58	136	62	8	19
2	882	68	212	209	58	58	141	66	12	19
6	885	67	214	205	65	59	146	78	16	19
10	890	66	213	203	68	58	140	75	18	19
20	896	66	215	205	84	55	160	97	31	19
100	854	63	278	214	461	36	602	486	174	23

¹ CM = calendula meal.

² DM = dry matter; CP = crude protein; AA = amino acids; CF = crude fibre; EE = ether extract; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; GE = gross energy.

Table 3. Amino acid composition (% of feed protein) of calendula meal and Calendula meal containing diets fed to young pigs.

Amino acid	% calendula meal in diet					Calendula meal
	0	2	6	10	20	
Aspartic acid	9.6	9.6	9.5	9.4	9.5	8.0
Threonine	4.3	4.2	4.0	4.0	4.0	3.2
Serine	6.0	5.7	4.9	5.0	4.8	3.9
Glutamic acid	18.0	18.0	17.7	17.6	17.6	14.7
Glycine	5.2	4.9	4.5	4.6	4.5	4.8
Alanine	5.2	5.1	4.8	4.8	4.8	3.8
Valine	5.2	5.1	5.1	5.0	5.0	4.4
Isoleucine	4.3	4.4	4.3	4.2	4.3	3.6
Leucine	8.4	8.5	8.3	8.2	8.3	5.6
Tyrosine	4.0	4.0	4.0	3.8	3.8	2.5
Phenylalanine	5.0	5.0	4.9	4.8	4.9	3.9
Histidine	2.7	2.4	2.5	2.5	2.3	2.3
Lysine	6.2	6.0	6.0	5.9	6.0	3.2
Arginine	5.8	5.8	5.8	5.7	6.0	6.2
Proline	6.3	6.3	6.0	6.0	5.8	3.2
Cystine	1.8	1.8	1.8	1.7	1.7	1.8
Methionine	1.9	1.9	1.9	1.9	1.9	1.7
Total	99.8	98.8	96.0	95.2	95.3	76.8

% of feed protein

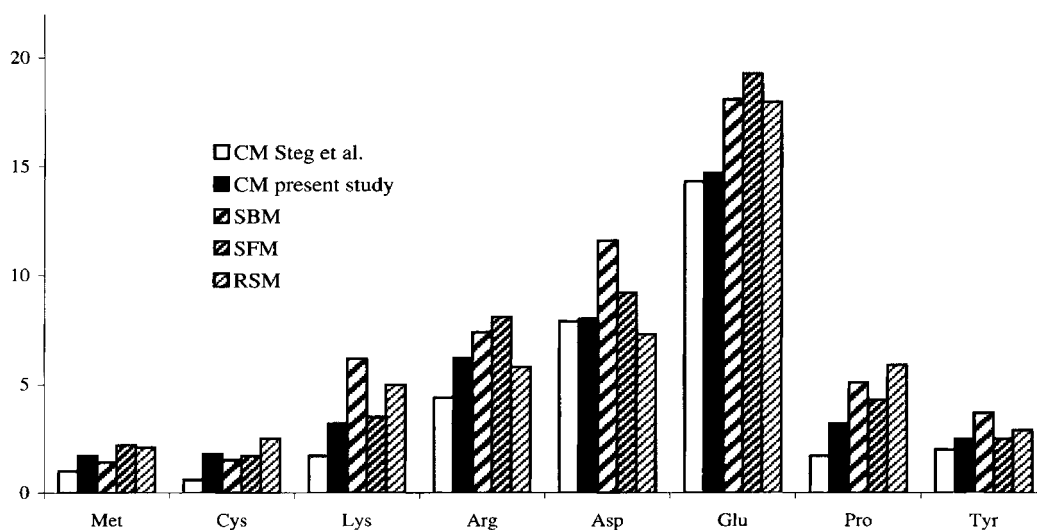


Figure 1. Amino acid composition (% of feed protein) of different feed components. Arg = arginine; Asp = aspartic acid; Cys = Cystine; Glu = glutamic acid; Lys = lysine; Met = methionine; Pro = proline; Try = tryrosine. CM = calendula meal; SBM = soya bean meal; SFM = sunflower seed meal; RSM = rapeseed meal.

2000). CM contained more non-amino acid nitrogen than the replaced ingredients, and similar (soya bean by-products) or slightly lower (sunflower seed meal) percentages of methionine and cystine. Lysine content was lower than expected and considerably lower than the levels of lysine listed in the Dutch Feed Table (Anon., 2000) for soya bean by-products.

Animal performance

On the whole, the animals remained healthy and performed well. Incidentally, light cases of diarrhoea occurred. Diarrhoea was observed with 4 animals of the 6% CM treatment for one day in the third week and with 4 animals of the 20% CM treatment for 5 days during the first week of the trial. The second incidence could be an indication of bowel irritation resulting from adaptation to the high level of CM in the diet. As the animals responded well to treatment with penicillin it was concluded that the animals were suffering from a mild enteritis infection unrelated to the feeding of CM. The performance data from these animals were included in the analysis of the results.

There were 5 mild incidences of lameness involving 3 piglets fed 20% CM and 2 fed the control diet (0% CM). The animals recovered within a week after treatment with penicillin. However, one animal fed 6% CM was persistently lame throughout the trial; its results were excluded from the analyses. These incidences of lameness were not related to the treatments.

One animal of the 20% CM treatment remained a poor performer throughout the experiment. During the first week of the trial the piglet concerned was treated for an ear infection. The data from this animal were not included in the analyses.

Average bodyweights during the trial are summarized in Table 4. At the beginning of the trial, the average weights did not differ significantly between treatments. At the end of the trial, the piglets that had been fed a 2% or a 6% CM diet were significantly heavier than those fed the control or other diets.

The changes in bodyweight over the various intervals show significant differences during the last week of the trial. The animals fed a 2% or 6% CM diet displayed a significantly larger (1.3 to 1.5 kg) bodyweight increase than those fed a CM-free diet. During the last week of the trial, average bodyweight increase was significantly

Table 4. Effect of calendula meal containing diets on average bodyweight (kg) during the feeding trial.

Diet (% CM) ¹	Days after beginning the trial			
	1	17	30	37 ²
0	18.1±2.2	28.9±3.1	37.8±4.0	44.0a±4.4
2	17.8±1.5	29.7±2.7	39.2±4.0	46.9b±4.7
6	18.8±2.2	30.3±3.2	39.9±4.1	47.5b±4.8
10	17.2±1.5	27.9±3.5	37.3±3.0	44.1a±3.6
20	17.3±1.6	28.6±3.0	37.7±3.3	42.9a±3.9

¹ Percentage calendula meal in the diet.

² Averages followed by a different letter are statistically different ($P < 0.10$).

Table 5. Effect of calendula-meal containing diets on average daily weight gain per animal, daily feed consumption per group and feed conversion ratio.

Diet (% CM) ¹	Daily gain per animal ² (kg)	Daily feed consumption per group ² (kg)	Feed conversion ratio ³
0	0.701a	10.5a	1.824
2	0.788b	11.8c	1.828
6	0.777abc	10.8b	1.759
10	0.727ac	10.7b	1.730
20	0.692ad	9.8a	1.875

¹ Percentage calendula meal in the diet.

² Averages in the same column followed by a different letter are statistically different ($P < 0.10$).

³ Ratio expressed as kg feed needed per kg growth of the group.

lowest for the 20% CM diet. However, during the experiment as a whole, the animals fed a 2% or 6% CM diet grew significantly better than those fed a CM-free diet. There were no significant differences in bodyweight increase between the control animals (0% CM) and the animals fed 10 or 20% CM.

Table 5 summarizes the average daily weight gain and feed consumption as recorded for each diet and the feed conversion ratios calculated from these data. Average daily gain in live weight for animals fed a 2% CM diet was significantly higher than for the ones fed a diet without CM. Daily gain per animal and daily feed consumption tended to decrease with a higher percentage CM in the diet. This suggests that CM had a negative influence on palatability. Obviously, this reduction in feed intake influenced live weights.

Table 6 presents the levels of various substances that were measured in the pigs' blood. Differences in haematocrit and haemoglobin levels between treatments were very small. The observed haematocrit levels were within the reference limits (26–41%; Friendship *et al.*, 1984). Small differences between treatments were also found for haemoglobin, but these levels remained below the expected range (9–14 mm l⁻¹) for weaned pigs (Friendship *et al.*, 1984). For all treatments (including the CM-free diet) the levels of oxygenated haemoglobin increased during the trial, but the individual data were subject to large variations.

Creatinine levels (Table 6) also serve as an indicator of kidney function. The data show similar patterns for all treatments. Observed increases during the trial were largest for the 0% CM treatment, but the levels remained within the reference limits (0.67–1.72 mm l⁻¹, Friendship *et al.*, 1984).

Mean levels of aspartate-aminotransferase in the serum taken from animals of the 2% and 6% treatments (Table 6) showed considerable but not significant increases during the trial. Generally speaking, the levels remained within the range (21–94 U l⁻¹) considered acceptable for weaning pigs (Friendship *et al.*, 1984).

Also the alanine-aminotransferase levels (Table 6) remained within the range (8–46 U l⁻¹) considered acceptable for healthy weaners (Friendship *et al.*, 1984). No significant differences were observed between treatments.

PERFORMANCE OF YOUNG PIGS FED MARIGOLD MEAL

Table 6. Effect of calendula meal containing diets on the average level of blood parameters of young pigs at the beginning (day 1) and at the end (day 37) of the feeding trial. Standard deviations in parentheses.

Day no.	% calendula meal in diet					LSD ¹	
	0	2	6	10	20	0.10	0.05
Haematocrit (%)							
1	35.4(1.74)	35.2(1.90)	35.8(1.76)	35.1(2.13)	35.7(1.75)	1.20	1.44
37	35.5(2.39)	37.1(1.87)	36.7(1.51)	34.8(2.62)	34.9(2.19)	1.31	1.56
Haemoglobin (mm l ⁻¹)							
1	7.2(0.36)	7.2(0.36)	7.3(0.42)	7.1(0.50)	7.0(0.45)	0.29	0.35
37	7.3(0.50)	7.6(0.34)	7.5(0.33)	7.1(0.59)	7.2(0.52)	0.29	0.34
Oxygenated haemoglobin (%)							
1	50.7(23.14)	47.7(25.85)	39.7(18.12)	46.3(27.88)	45.1(27.47)	14.5	17.4
37	57.3(21.12)	56.2(15.18)	62.2(19.40)	56.1(16.03)	59.7(15.98)	10.6	12.7
Creatinine (mm l ⁻¹)							
1	0.076(0.0115)	0.071(0.0110)	0.076(0.0109)	0.076(0.0138)	0.073(0.0101)	0.0075	0.0090
37	0.094(0.0110)	0.086(0.0080)	0.089(0.0083)	0.083(0.0104)	0.088(0.0071)	0.0054	0.0065
Aspartate-aminotransferase (U l ⁻¹)							
1	49.5(35.52)	38.9(15.20)	38.6(14.85)	48.7(16.97)	37.9(17.50)	18.34	21.96
37	43.8(19.16)	76.3(48.97)	64.8(30.45)	51.3(28.50)	39.5(10.77)	12.73	15.24
Alanine-aminotransferase (U l ⁻¹)							
1	33.4(6.92)	34.4(6.60)	31.5(5.18)	36.0(9.80)	34.7(7.46)	9.75	11.68
37	333(5.24)	39.0(6.75)	37.6(7.07)	35.0(7.24)	35.4(5.41)	3.93	4.71
Lactate dehydrogenase (U l ⁻¹)							
1	1359(563.6)	1158(324.4)	1233(253.2)	1216(363.4)	1182(349.2)	237.8	284.8
37	1231(517.0)	1324(482.1)	1297(350.2)	1235(350.2)	1143(390.0)	212.4	254.4
Zinc (mm l ⁻¹)							
1	0.56(0.15)	0.51(0.09)	0.52(0.10)	0.53(0.09)	0.50(0.13)	0.067	0.080
37	0.70(0.12)	0.77(0.13)	0.69(0.15)	0.66(0.11)	0.68(0.13)	0.069	0.082

¹ Least significant differences at $P < 0.10$ and $P < 0.05$.

Individual lactate dehydrogenase levels varied between approximately 900 and 1700 U l⁻¹. Despite this wide range, no significant differences were found between treatments (Table 6).

Zinc was measured in the blood serum because it provides a guide to the general health status of the animals (Table 6). This trace element plays an important role in animals, a shortage leading to health problems and reduced performance (Anon.,

Table 7. Effect of calendula meal containing diets on average¹ weight (expressed in grams and as % of bodyweight) of vital organs determined at *post mortem* examination.

Diet (% CM) ²	Heart	Liver	Kidneys	Thyroid	Pancreas	Heart	Liver	Kidneys	Thyroid	Pancreas
	(g)					(% of bodyweight)				
0	183ab	1105ab	171a	4.66a	98ab	0.42a	2.53	0.419a	0.0106a	0.225a
10	188a	1216a	223b	4.50ab	102a	0.38b	2.44	0.448ab	0.0091b	0.203b
20	176b	1025b	196a	3.89c	91b	0.43a	2.50	0.475b	0.0095b	0.219ab

¹ Averages in the same column followed by a different letter are statistically different ($P < 0.10$).

² Percentage calendula meal in the diet.

1998a). No significant differences were observed between treatments.

Post mortem weights of the organs taken from animals fed the 0%, 10% or 20% CM diet differed significantly between treatments (Table 7). The pigs fed a 20% CM diet had a significantly lighter liver and thyroid gland than the ones on a CM-free diet, whereas the pigs on a 10% or a 20% CM diet had significantly heavier kidneys. This can be seen as an indication of increased kidney activity for the animals fed a 10% CM diet. However, if organ weights are expressed as percentages of bodyweight the differences become less pronounced.

The hearts from the 10% CM group were on average significantly lighter than the hearts from the 0% and 20% CM groups. The thyroid glands from the pigs that had been fed a CM-containing diet were significantly lighter than the thyroid glands from the CM-free group. In the 10% CM group the average weight of the pancreas in relation to bodyweight was significantly lower than in the 0% CM group.

Detailed histo-pathological examination of the liver, kidney and thyroid gland of 16 control (0% CM) pigs and 16 pigs on a 20% CM diet, displayed light inflammation in the liver from one animal in the control group and from 2 in the 20% CM group. No clear degenerative changes were observed. In 11 livers from pigs in the control group and 5 livers from the 20% CM treatment a mild activation of the Kupfer cells was found. This was considered due to an unspecified inflammation, unrelated to the treatments.

Closer examination of the kidneys revealed no changes in the epithelia of any of the animals. In 9 pigs of the 0% CM and 6 of the 20% CM group an inflammation of the pyelum was detected, which was thought to be the result of an unspecified infection unrelated to the treatments. No changes were observed in the thyroid tissue from any animals.

Conclusions

Young pigs that were fed a 2% CM diet consumed significantly more feed than animals fed a CM-free diet. Feed intake tended to decline with increasing CM content in the diet. With increasing CM content, lignin content of the diet increased significantly. This could explain the lower intake of the pigs fed a 20% CM diet as observed during the first 17 days of the experiment.

Average bodyweight gain was highest for animals fed a 2% CM diet and lowest for animals fed a 20% CM diet. The animals that were fed a 2%, 6% or 10% CM diet tended to grow better than the ones fed a 0% or a 20% CM diet.

Slightly lower liver weights were recorded for the pigs fed a 10% CM diet, and slightly higher kidney to body weight ratios were observed for the pigs that had been fed a 10% or a 20% CM diet.

The blood parameters measured gave no rise for concern: the general patterns displayed no significant differences and remained within the ranges of reference.

It is concluded that calendula meal has potential as feed ingredient for pigs. But caution should be taken not to include more than 10% of it in pig diets.

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