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EFFECTS OF CHROMIUM YEAST SUPPLEMENTATION ON GROWTH PERFORMANCES AND MEAT QUALITY IN RABBITS

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Abstract: The aim of the trial was to estimate the effect of dietary Cr-yeast addition to growing rabbit diet on growth performance, meat composition, muscle fatty acid profile and Cr content of meat and edible organs. Ninety-six male rabbits were weaned at 35 days and divided into 4 groups (T1, T2, T3 and T4) of 24 each. The animals were fed *ad libitum* for the whole trial (44 days) with pelletted diets differing in the presence of yeast (*Saccharomyces cerevisiae*), grown or not on Cr-enriched medium. The control diet (T1) did not contain yeast, the T2 diet was supplemented with non-enriched yeast, while the T3 and T4 diets were supplemented with Cr(III)-enriched yeast to increase the concentration of Cr by 0.400 mg/kg and 0.800 mg/kg, respectively. Control diet (T1) contained 0.830 mg/kg Cr due to the presence of the trace element in raw materials. The dietary treatment did not affect either the mortality, or the growing and slaughtering performance, or the incidence of kidneys, scapular and perirenal fat on carcass weight. A reduction in the liver incidence on the carcass was observed in the T3 group compared to the T4 (4.36% *vs* 5.67%; $P<0.01$). Hind leg proportion on carcass weight and its muscle to bone ratio, as well as chemical, physical and organoleptic characteristics of meat did not differ among groups. The presence of Cr(III) in the feed did not alter the fatty acid profile of muscular fat or the chemical composition, pH and colour of the meat. The Cr concentration in meat and edible organs (liver and kidneys) was not affected by treatment. In conclusion, Cr-yeast supplementation had no positive effects on the rabbit growth performance and carcass and meat quality and did not increase the Cr(III) content of meat for human consumption.

Key words: growing rabbits, chromium, yeast, performance, meat quality.

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INTRODUCTION

Trivalent chromium [Cr(III)] plays a primary role in the animal metabolism as a constituent of the Glucose Tolerance Factor (GTF), an enzymatic complex able to increase insulin activity (SCHWARZ and MERTZ, 1959).

In addition, Cr(III) seems to influence the lipogenic activity of the organism, modifying the amount of fat deposits in monogastric animals. Several research showed the ability of some forms of organic Cr - in particular Cr picolinate (CrPic)- to reduce the fatness of the pig carcass (PAGE *et al*., 1993; LINDEMAN *et al*., 1995; MOONEY and CROMWELL, 1995, 1997; BONOMI *et al*., 1997a).

The effectiveness of Cr(III) addition to diet is attributed to the supposed lack of this trace element in diets formulated for growing animals. As a matter of fact, Cr(III) was recently included in the list of the essential trace elements for pigs, even if the minimum requirements were not indicated for this species (CROMWELL, 1999).

Cr(III) addition to feeds for livestock is usually proposed in this context. It has to be pointed out that often experimental results do not agree with each other, probably as a consequence of the trace element content in the raw materials. In a recent work, we investigated the effects of Cr(III) on growing rabbits (LAMBERTINI *et al*., 2000) but the results were discordant with those obtained by other authors on the same species (BONOMI *et al*., 1999; CUTRIGNELLI *et al*., 1999). Starting from this work we judged it useful to further investigate the effectiveness of supplementing growing rabbit feed with Cr-yeast, taking into consideration not only productive performances, carcass composition and meat parameters but also fatty acid profiles of muscle. Moreover, the Cr content was determined for meat and edible organs, as they make for possible alimentary sources of the trace element for humans.

MATERIALS AND METHODS

The trial was carried out in a commercial farm using 96 male hybrid rabbits. At weaning (35±3days), the animals were homogeneously divided into 4 groups of 24 individuals each, according to criteria accounting for weight and litter, and housed in bicellular cages (28 x 41 x 28-h cm). During the trial (44 d), the control group rabbits were fed *ad libitum* with a commercial pelleted feed (diet T1). Rabbits in the other groups were fed with the same diet supplemented before mixing and pelleting with lyophilised yeast (*Saccharomyces cerevisiae*), enriched -or not- with Cr(III), according to the Table 1.

The T2 group was used to make sure that any modification in the considered parameters would be due to the Cr(III) addition and not to yeast. The T3 diet was prepared by mixing the T1 and T4 pelletted feeds (0.5 T1 + 0.5 T4). The chemical composition and Cr concentration of the experimental feeds are reported in Table 2. The Cr content of the yeasts used as supplement of the feeds (T1, T2 and T4) was determined by inductively coupled plasma atomic emission spectrometry, following the same analytic procedure used for biological samples, and described later on.

The Cr concentration of the supplemented yeast was determined in 214 mg/kg for the high-Cr yeast and 3.2 mg/kg for the normal yeast. These values agreed with those declared by the producing firm.

Table 1: Yeast and Cr addition to experimental diets.

Diets	Normal yeast	High-Cr yeast*	Cr addition
T1(control)	-	-	-
T2	400 g/100 kg	-	-
T3 (1/2 T2+1/2 T4)	-	200 g/100 kg	0.400 mg/kg
T4	-	400 g/100 kg	0.800 mg/kg

* Cr concentration of high-Cr yeast declared by the producing firm was 200 mg/kg.

Individual weight and cage feed consumption (two rabbits per cage) were recorded every 14 days, in order to calculate the daily weight gain and the feed efficiency ratio. The number of dead animals for each treatment was also recorded. At the end of the fattening period, all the rabbits, 79-days old, were slaughtered in a commercial slaughterhouse. The weights of the carcasses (including head, heart, lungs, liver, kidneys, and scapular and perirenal fat deposits), hot and after cooling at 0°C for 1.30h, were recorded.

In 40 carcasses (10 representative per group), the pH at 45 min post mortem (pH_{45}) of the *longissimus dorsi* muscle measured between the 6th and the 7th lumbar vertebra was measured, as well as the ultimate pH (pHu) after refrigeration at 4°C for 24 hours (OUHAYOUN and DALLE ZOTTE, 1996). Moreover, meat colour parameters were measured on the cross-section of the same muscle on the right side, according to the CIELab system (L*, a*, b*, Chroma*, Hue°), using a Hunterlab MiniScan/ XE™.

Table 2: Chemical composition of the experimental diets (% as fed).

	Diet T1	Diet T2	Diet T4
Dry matter	91.0	91.3	90.8
Crude protein	16.1	16.1	16.
Ether extract	2.9	2.5	2.6
Crude fibre	13.2	12.9	12.7
Ash	9.5	9.4	8.9
Digestible energy [1] (MJ/kg)	10.6	10.7	10.7
Neutral detergent fibre	33.1	32.6	30.8
Acid detergent fibre	19.1	19.0	18.0
Acid detergent lignine	4.6	4.2	4.5
Chromium (mg/kg)	0.830	0.855	1.520

Ingredients of the commercial basal diet: dehydrated lucerne meal, soft wheat middling, soft wheat bran, sunflower meal, sugarbeet pulp, barley meal, soybean meal, vegetal oil, sugarcane molasses, calcium carbonate, dicalcium phosphate, sodium chloride, DL-methionine, minerals and vitamins, robenidine (66 mg/kg).
[1] Calculated (PARIGI BINI and DALLE RIVE, 1977).

Liver, kidneys, scapular and perirenal fat and left hind leg from the same carcasses were then separated and weighed. The left hind leg was dissected and its bone components were separated from the meat to determine the meat/bone ratio, representative of the whole carcass (Blasco and Ouhayoun, 1996). The edible tissues of the hind leg were analysed to determine water, protein, and lipid concentration (AOAC, 1995). Lipids were also extracted from the same muscle following the method suggested by Bligh and Dyer (1959). From the extract, the fatty acid profile of muscular lipids was determined, after performing cold methilation of fatty acids with the technique proposed by Fraga and Lercker (1984). This determination was accomplished by gas chromatography, using a capillary column CP SIL 88 WCOT FUSED SILICA (100m length, 0.2mm film thickness, 0.25mm inner diameter), working from 172°C to 220°C (8°C/min) for a total of 30 min, with injector and detector at 250°C, and using a ionised flame detector.

Finally, samples of liver, kidney, and *longissimus dorsi* muscle from the same 40 carcasses were analysed for total Cr concentration. Sampling was executed with plastic instruments, then tissues were mineralised by microwave assisted acid digestion with nitric acid and hydrogen peroxide mixture in a closed vessel system. The determination of Cr concentration was done by means of a sequential spectrophotometer ICP-AES (EPA Method 3052, 1996; Miller-Ihli, 1996).

All the data were analysed by one-way analysis of variance, using the GLM procedure of the SAS statistical package (SAS, 1989).

When the F test analysis of variance was significant ($P<0.05$), differences among means were compared using the SNK test. Mortality rates among the different groups were compared using the χ^2 test.

RESULTS AND DISCUSSION

The Cr concentration of the control diet was 0.830 mg/kg, a similar value to

that obtained in our previous research (LAMBERTINI *et al*., 2000), confirming the presence of the trace element in raw materials used for manufacturing commercial feeds. So the other yeast supplemented diets had higher Cr concentration (0.855 mg/kg and 1.52 mg/kg for T2 and T4 respectively).

The overall mortality was 7.3%, an acceptable value for the considered productive phase. In particular, no animal died in the group receiving Cr at the highest concentration (T4 group), while 3 animals (12.5%) died in T2 group, and 2 (8.3%) in both T1 and T3 groups. Although considering the small number of the animals, the mortality did not differ significantly among the experimental treatments ($\chi^2 = 4.57$; P=0.207), thus confirming the results of the previous study (LAMBERTINI *et al*., 2000) and those reported by other authors (BONOMI *et al*., 1999; CUTRIGNELLI *et al*., 1999).

Live weight, daily gain and feed intake are reported in Table 3. As in the previous trial (LAMBERTINI *et al*., 2000), the final live weight of all the rabbits were proportional to slaughter age and adequate for the performances normally reached in the farm where both trials were carried out.

Growth performance was not affected by the treatments, independently of the presence in the feeds of yeast grown on Cr-enriched medium and from the supplement concentration. These results confirm those of a previous work (LAMBERTINI *et al*., 2000), where no improvement in productive performances was detected, even with significantly higher Cr supplement levels (1.6 mg/kg). Also CUTRIGNELLI *et al*. (1999) did not find any effect of high Cr-yeast on growing rabbits, although not working in optimal temperature conditions. On the other hand, BONOMI *et al*. (1999) obtained meaningfully higher productive results in rabbits receiving feed with 0.4 or 0.6 mg/kg of Cr(III) from enriched yeast. Among monogastric animals, most experimental evidence refers to pigs. In general, independently of the form and concentration of the supplement, results obtained by different authors were not homogeneous, as already discussed (LAMBERTINI *et al*., 2000).

Table 3: Growth performance and slaughtering data.

	Experimental groups				RSD	*P*-value
	T1	T2	T3	T4		
Rabbits (n)	22	21	22	24		
Liveweight at weaning (g)	1184	1184	1185	1183	77	0.99
Liveweight at slaughter (g)	2603	2636	2533	2595	205	0.42
Daily weight gain (g/d)	32.3	32.7	30.7	32.1	4.4	0.47
Daily feed intake [1] (g/d)	110.3	104.3	105.3	107.2	11.2	0.28
Feed conversion ratio [1]	3.46	3.55	3.60	3.35	0.22	0.07
Hot carcass weight (g)	1586	1605	1553	1597	137	0.60
Dressing percentage (%)	60.9	60.9	61.3	61.5	1.7	0.56

RSD: Residual standard deviation.
[1]Cage with two rabbits; mean value for one rabbit.

The disagreement among experimental results may be due to the different level of Cr in the raw materials used to prepare the feeds (BONOMI *et al*., 1997; GIRI *et al*., 1990; KUMPULAINEN, 1992). Nevertheless, it cannot be excluded that the absence of positive results reported by several authors, and also for this trial in groups T3 and T4, could be due to the fact that the animals were not in a state of Cr deficiency. It has to be pointed out that this condition represents the usual situation where feed enrichment with organic Cr is proposed.

According to WRIGHT et al. (1994), a Cr deficiency could occur as a consequence of stressful conditions, which was not the case in this trial, anyway. However, more recent research does not support these observations. In fact, no positive effect due to Cr supplementation was observed in pigs subjected to experimental stress, such as reduced and inadequate cage space (WARD *et al*., 1997), or the assumption of *Escherichia coli* lipololysaccharides (VAN HEUGTEN and SPEARS, 1997).

Only under “high stress conditions” (intravenous injection of ACTH), which

can not be easily observed in the farm, the supplementation of Cr to piglets seemed to cause a better peripheral glucose utilisation (BALDI *et al.*, 1999).

Slaughtering data and carcass (Tables 3 and 4) and meat quality (Table 5) were not modified by the feeding treatments. On the contrary, the liver incidence (Table 4) was significantly lower ($P<0.01$) in rabbits of group T3 than in rabbits receiving the feed at the highest Cr concentration (T4). This result can not be easily explained, especially as the difference is between T3 and T4 groups, not between T3 and control. Therefore we believe that this result cannot be ascribed to the treatment with a sufficient degree of certainty. Also BONOMI *et al.* (1999), who reported better growth performance for rabbits receiving Cr-yeast, did not find any effect on the weight of the main organs.

Further recordings regarding meat quality, i.e. pH and colour, are shown in Table 6. The treatments did not alter meat colour or its *post mortem* acidification.

Also the fatty acid profile of the hind leg muscular fat and the ratio between saturated to unsaturated fatty acids (Table 7) did not show any modification.

Table 4: Carcass characteristics.

	Experimental groups				RSD	*P*-value
	T1	T2	T3	T4		
Carcasses (nº)	10	10	10	10	-	-
Cold carcass weight (CC) (g)	1576	1608	1523	1580	129	0.521
Liver (% CC)	4.97^{ab}	5.03^{ab}	4.36^{b}	5.67^{a}	0.74	<0.01
Kidneys (% CC)	0.88	0.92	0.90	0.88	0.16	0.93
Scapular fat (% CC)	0.31	0.54	0.40	0.39	0.19	0.07
Perirenal fat (% CC)	1.36	1.40	1.45	1.36	0.34	0.94

RSD: Residual standard deviation.

Table 5: Hind leg composition.

	Experimental groups				RSD	*P*-value
	T1	T2	T3	T4		
Observations	10	10	10	10	-	-
Hind leg incidence [1] (%CC)	14.04	13.96	13.92	13.76	0.58	0.73
Hind leg meat to bone ratio	6.06	5.88	5.61	5.97	0.96	0.75
Meat chemical composition						
water (%)	74.81	74.15	74.03	74.10	1.37	0.56
protein (%)	21.19	21.56	20.93	21.01	0.98	0.42
lipids (%)	2.54	2.30	2.31	2.55	0.854	0.85

RSD: Residual standard deviation.
[1]on the cold carcass weight

Table 6: Meat pH and colour.

	Experimental groups				RSD	*P*-value
	T1	T2	T3	T4		
Samples (n)	10	10	10	10	-	-
pH_{45}	6.87	6.85	6.88	6.83	0.17	0.58
pHu	5.85	5.90	5.83	5.83	0.15	0.66
Colour:[1]						
L*	41.8	42.7	43.6	42.8	2.9	0.62
a*	-3.25	-3.46	-3.27	-3.41	0.50	0.73
b*	0.54	0.90	1.15	0.88	0.88	0.50
C*	3.48	3.69	3.54	3.58	0.45	0.75
H°	-9.25	-15.29	-18.92	-14.61	14.86	0.55

RSD: Residual standard deviation.
[1]On *longissimus dorsi* muscle cross section between 6[a] and 7[a] lumbar vertebra

Many experimental results on Cr effects on carcass and meat quality refer again to pigs, as discussed in our previous work (LAMBERTINI *et al.*, 2000). LIEN *et al.* (2001) observed a decrease in backfat thickness and an increase in the loin-eye

Table 7: Fatty acid composition of the extracted fat of hind leg muscle.

	Experimental groups				RSD	*P*-value
	T1	T2	T3	T4		
Samples (n)	10	10	10	10	-	-
C12:0	0.43	0.45	0.49	0.46	0.18	0.92
C14:0	2.92	3.18	2.98	3.36	0.55	0.28
C15:0	0.62	0.66	0.67	0.63	0.07	0.29
C16:0	31.33	31.53	31.05	32.72	1.96	0.26
C16:1	3.23	3.50	3.58	4.12	1.17	0.40
C17:0	0.69	0.71	0.71	0.67	0.08	0.67
C18:0	8.50	8.54	8.43	7.80	1.02	0.33
C18:1	24.99	25.00	24.47	26.24	2.16	0.32
C18:2	21.37	20.49	21.24	19.11	2.36	0.14
C18:3	2.45	2.87	2.69	2.70	0.63	0.53
C20:3	0.42	0.36	0.42	0.28	0.18	0.26
C20:4	3.06	2.47	3.26	1.91	1.60	0.24
SFA	44.49	45.07	44.33	45.64	2.15	0.52
MUFA	28.22	28.74	28.06	30.36	3.12	0.35
PUFA	27.29	26.18	27.61	24.00	3.62	0.13
SFA/MUFA	1.59	1.59	1.61	1.51	0.80	0.64
SFA/PUFA	1.66	1.75	1.65	1.93	0.30	0.16

RSD: Residual standard deviation.
SFA: saturated fatty acids, as C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0.
MUFA: monounsaturated fatty acids, as C16:1 + C18:1.
PUFA: polyunsaturated fatty acids, as C18:2 + C18:3 + C20:3 + C20:4.

area, but on the other hand they noticed an increase in the adipocyte lipogenic rate and a tendency towards enlarged adipocytes volume. Analogous results for the carcass structure were obtained by Xi *et al*. (2001), who ascribe to Cr the ability to increase the activity of hormone sensitive lipase in subcutaneous fat, promote muscle anabolic metabolism and inhibit the activity of lipogenic enzymes. Interestingly in rats, the effectiveness of Cr picolinate supplementation seems to be correlated to the genetic line. In particular, a reduction in body fat level seems to occur in animals with lower growing potential and reduced ability to develop lean body mass (Hasten *et al*., 1997). Nevertheless, recent studies by Mooney and Cromwell (1999), carried out on pigs with different lean gain potential, did not show any interaction between genetic line and feed treatment with Cr.

Also our results concerning carcass composition and fatness were not affected by Cr-yeast addition. The fact that the amount of perirenal fat did not increase, which does not confirm previous observations of ours (Lambertini *et al*., 2000), may suggest that this feature is not related to Cr(III) supplement.

Even for the chemical composition of the hind leg muscle, and in particular the muscular fat content, no effect due to the treatments was detected, in substantial agreement with what was reported by Bovera *et al*. (1999). Also the fatty acid profile of muscular fat, and in particular the ratio between saturated, unsaturated, and polyunsaturated fatty acids, were not modified by the feed treatments. In pigs, Lien *et al*. (2001) report an increased saturated fatty acid level in adipose tissue probably ascribable to the indirect stimulating effect of Cr on lipogenic rate, and in particular on the *de novo* saturated fatty acid synthesis, which could cause a decrease in unsaturated fatty acids for "dilution effect". Lemme *et al*. (1999) did not observe any modification in the fatty acid composition, except for a linear increase in palmitoleic acid, in growing-finishing pigs. Also our experimental results did not show any effect of high Cr yeast on fatty acid composition.

Again, as for the *post mortem* acidification ability and the colour parameters of meat, no meaningful difference among the experimental groups was found. The

Table 8: Chromium concentration of meat *(longissimus dorsi muscle*) and edible organs.

	Experimental groups				RSD	*P*-value
	T1	T2	T3	T4		
Samples (n)	10	10	10	10	-	-
Liver (μg/kg)	88.6	81.4	95.5	73.3	20.3	0.11
Kidneys (μg/kg)	70.87	75.04	77.84	67.79	19.15	0.07
Meat (μg/kg)	43.23	34.02	39.10	32.83	9.70	0.08

RSD: Residual standard deviation.

absolute values were similar to those obtained in our previous trial (LAMBERTINI *et al*., 2000), confirming a lack of effects on glucose uptake by the muscle.

In the end, the concentration of Cr in edible organs (kidneys and liver) and in the *longissimus dorsi* muscle (Table 8) was also found to be unaffected by the feeding treatment. Our results do not agree with those of other authors (BONOMI *et al*., 1999; BOVERA *et al*., 1999), who always found a higher Cr concentration in the tissues of treated animals, sometimes proportional to the supplementation level (BONOMI *et al*., 1999). The results by these researchers are different -also in absolute values- from those obtained in this experience, especially for liver and kidneys. However, it must be emphasised that the biological sample preparation techniques for the dosage of the trace element, involving grinding and homogenisation with a steel blade, may cause an enrichment in metallic Cr. Furthermore, the lack of differences among the treated groups should be explained considering that Cr absorption is in inverse relation to its food content, at least in humans (ANDERSON, 1987).

CONCLUSIONS

The results of this trial confirm that health conditions and growth performances are not affected by Cr supplementation.

Moreover, independently of the supplementation level, organic Cr does not seem to have an effect on dressing percentage, meat to bone ratio of the carcass, meat chemical composition, and its *post mortem* pH, and colour. Chromium addition does not modify either the amount of scapular and perirenal fat deposits, or the fatty acid profile of muscular fat. A dietary enrichment with Cr does not increase its concentration in edible organs and meat.

It can be concluded that working at conditions usually found in commercial farms the supplementation of Cr (III) via enriched yeast in rabbit feeds does not result in any significant improvement of production values and does not seem to be an effective way to increase the Cr content in animal products for human consumption.

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