Endocrine Response of Hybrid Rabbits of Different Ages and Under Two Environmental Temperature Conditions

G. M. Chiericato*, Chiara Rizzi*, C. Boiti**, C. Canali**. Vania Rostellato*

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Summary

The trial was carried out on 44 hybrid male rabbits in order to study the plasma levels of testosterone (T), dihydrotestosterone (DHT), cortisol (C), triiodothyronine (T_3) and thyroxine (T_4) in relation to age (71 vs 85 days) and environmental temperature (20 vs 27°C). Age did not affect T, DHT, T+DHT. Only T/DHT resulted lower (P<0.01) for older rabbits. The 71 and 85 day-old rabbits showed similar levels of C, T_3, T_4 and T_4/T_3. The different thermal levels did not influence T, DHT, T+DHT, T/DHT and C. The rabbits reared at 27°C showed similar values of T_3 and T_4 but a higher (P<0.05) T_4/T_3 ratio in comparison with those kept at 20°C. Digestible energy intake/metabolic weight appeared to influence (P<0.01) T3 positively and T_4/T_3 negatively.

Résumé

L'essai a été réalisé sur 44 lapins mâles hybrides afin d'étudier les niveaux plasmatiques de testostérone (T), dihydrotestostérone (DHT), cortisol (C), triiodiothyronine (T_3), et thyroxine (T_4) en fonction de l'âge (71 vs 85 jours) et des conditions thermiques d'élevage (20 vs 27°C). L'âge n'a pas influencé les niveaux de T, DHT et de T+DHT. Seul le rapport T/DHT s'est révélé inférieur (P<0.01) au 2e prélèvement. Les lapins âgés de 71 et 85 jours ont présenté des valeurs identiques de C, T_3, T_4, et T_4/T_3. Les différentes valeurs thermiques n'ont pas influencé les taux de T, DHT, T+DHT, T/DHT et C. Les lapins élevés à 27°C ont présenté des valeurs semblables de T_3 et T_4, mais un rapport T_4/T_3 plus élevé (P<0.05). L'ingestion d'énergie digestible par unité de poids vif métabolique a influencé positivement (P<0.01) les niveaux de T_3 et négativement (P<0.01) le rapport T_4/T_3.

1. Introduction

The plasma endocrine status of rabbits has been studied in some trials in relation to physiological and exogenous factors. With regard to androgens, Berger *et al.* (1, 2. 3) studied the developmental pattern of plasma testosterone and dihydrotestosterone in growing rabbits. Correlations between testosterone and sexual and behavioural development were also found (3).

Ambient stress factors such as temperature (16, 21, 22) and noise (22) resulted to affect corticosteroid plasma levels. High environmental temperature (21) influenced the thyroid hormones as well as high doses of perchloric acid ammonia (8).

Our previous research considered the influence of age and nutritive level on androgens (5). Endocrine status was also studied in relation to different thermal conditions (4). Given the scarcity of experimental work on the effect of environmental temperature and age on the endocrine response in male rabbits. the aim of the present research is to provide an insight on the effect of these factors on androgen, corticosteroid and thyroid hormone plasma profiles.

2. Material and methods

Fourty-four Provisal male rabbits were used, characterized by a mean initial live weight of 989 g.

The animals were 35day-old commercial four-way crossbred type rabbits, raised under the same rearing conditions and nutritional plan during the pre and post weaning period. Commercial pelletted feed (crude protein=17.18% d.m.; crude fiber=5.48% d.m., digestible energy=10.57 MJ/kg as feed basis) was administered *ad libitum,* like for the water. The animals were randomly housed in two different rooms with rearing temperature levels of 20±1.94 and 27±1.23°C. Average relative humidity values were 75±7.21% for both temperatures. The animals were reared individually up to 85 days of age in a Californian battery cage system, without hindering ciecotrophy. The trial was carried out in autumn, so a heating system was used in the room at 27°C to reach this high temperature level. In the first room the ventilation was natural; the second was conditioned by an automatic control system for temperature and humidity. The photoperiod. provided by halogen lamps, consisted in 16 hours of light and 8 hours of darkness. with a light intensity

* Dipartimento di Scienze Zootecniche, Università di Padova. Agripolis. 35020 Legnaro - Padova - Italy
** Istituto di Fisiologia Veterinaria, Università di Perugia, Via S. Costanzo 4, 061000 Perugia - Italy
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Table I. Productive performance

		Age		Temperature		Error mean
		71 d	85 d	20°C	27°C	square*
Animals	n	20	24	24	20	
Initials BW	g	974	1005	995	983	3420
Final BW	g	2161^A	2643^B	2488^B	2316^A	36140
Metabolic BW	kg	1.40^A	1.57^B	1.51^B	1.45^A	0.0053
Weight gain	g/d	33.0	33.4	35.2^B	31.3^A	13.61
Feed intake	g/d	107^A	122^B	122^B	107^A	113
Feed efficiency	g/g	3.24^A	3.68^B	3.50	3.42	0.0605

Within a treatment, values assigned different superscript letter were significantly different (P<0.01).
*: 40 degrees of freedom.

Table II. Plasma levels of some hormones

		Age		Temperature		Error mean
		71 d	85 d	20°C	27°C	square*
Animals	n	20	24	24	20	
T	ng/ml	1.38	1.18	1.60	1.06	0.9922
DHT	ng/ml	0.60	0.79	0.80	0.59	0.2621
T/DHT		2.30^B	1.49^A	2.00	1.80	0.6146
T+DHT	ng/ml	1.98	1.97	2.40	1.65	2.1273
C	μg/dl	1.85	1.65	1.78	1.72	0.2386
T3	ng/ml	1.12	1.08	1.16	1.04	0.0701
T4	ng/ml	37.8	38.0	36.6	38.7	36.62
T4/T3		33.9	35.1	31.6^a	37.4^b	59.79

Within a treatment, values assigned different superscript letter were significantly different (A, B: P<0.01) (a, b: P<0.05).
*: 40 degrees of freedom.

of about 40 lux. Ammonia concentrations were similar between the two treatments, on average 8.5 ppm. Temperature and relative humidity values were constantly recorded by means of thermohygrographs (TIG - ITH -L.S.I).

At 71 (1st sampling) and 85 (2nd sampling) days of age, each rabbit was subjected to blood samplings at the same time in the morning after a fasting period of two hours. The blood was sampled from the auricular vein, collected in test tubes with 150 USP lithium heparin. centrifuged for 15 minutes at 3000 rpm and the plasma stored at -20°C until analysis. Testosterone (T) and dihydrotestosterone (DHT) plasma levels were quantified by radioimmunoassay (RIA) as described by Berger *et al.* (1). The specificity of the antiserum used [in the T-DHT RIA] was tested against related steroids and expressed as percentage of cross-reactivity. Only 5α-dihydrotestosterone had a significant cross-reactivity of about 50%, while for all the other androgens the cross-reaction was lower than 7% - 5α-Androstane-3α, 17βα diol (4%), 5α-Androstane-3β, 17βα diol (6.5%), 5β-dihydrotestosterone (1%), Epitestosterone (0.5%)-, and for both oestrogens and progestins lower than 0.05%. Cortisol (C), triiodothyronine (T_3) and thyroxine (T_4) plasma concentrations were determined using specific and standardized RIA procedures based on kits (Cambridge Medical Technology, USA) which have been validated for use in rabbits. The intra- and inter-assay coefficients of variation for each assay were 6.1-12.0%. All measurements were made in duplicate and only the values included in the limits of each method were retained.

Data were submitted to a two-way ANOVA with interaction, using the Harvey (10) package.

The correlations and regression equations between plasma hormone concentrations and some productive performance, daily gain (DG) and digestible energy intake (DEI) per unit of metabolic body weight (MBW) were calculated.

3. Results

As no significant interaction effects between age and temperature were observed, in the following tables only the main effects are reported.

Growth performances

Table 1 summarizes the productive performance exhibited by the tested rabbits. In relation to age, the 85 day-old rabbits exhibited significantly higher (P<0.01) final body (2643 *vs* 2161 g) and metabolic weights (1.57 *vs* 1.40 kg). Although the oldest rabbits presented higher feed intake (122 *vs* 107 g/d, P<0.01) the growth rate was similar between the groups (33.4 *vs* 33.0 g/d) resulting in a feed efficiency of 3.68 g/g in favour (P<0.01) of the younger animals (3.24 g/g).

As concerns rearing temperature effects, high thermal levels induced the animals to eat less (107 vs 122 g/d, P<0.01), thus presenting a significantly (P<0.01) lower daily body weight gain (31.3 *vs* 35.2 g). As a consequence final live weight (2316 vs 2488 g) and metabolic weight (1.45 vs 1.51 kg) were significantly lower (P<0.01) in comparison with the rabbits kept at 20°C. Feed efficiency did not differ between the 20 and 27°C groups, averaging 3.46 g/g.

Hormonal profiles

The plasma levels of some hormones are shown in table II. Concerning androgen levels no difference was found between animals of different age (85 *vs* 71 d): T (1. 18 *vs* 1. 3 8 ng/ml), DHT (0. 79 *vs* 0.60 ng/ml) and T+DHT (1.97 *vs* 1.98 ng/ml), but the T/DHT ratio appeared to be significantly lower (P<0.01) for the older rabbits (1.49 *vs* 2.30). All plasma androgen levels were similar between the two temperature groups, averaging 1.33, 0.70, 2.02 ng/ml for T, DHT and T+DHT, respectively. The T/DHT ratio did not differ (mean value: 1.90).

At increasing age plasma C concentrations showed a tendential decrease: 1.85 and 1.65 µg/dl in 71 and 85 day-old rabbits respectively. The rearing temperature did not affect plasma C concentrations (average: 1.75 µg/dl).

Going on to consider the thyroid hormones, age did not appear to influence the T_3 (1.12 vs 1.08 ng/ml) and

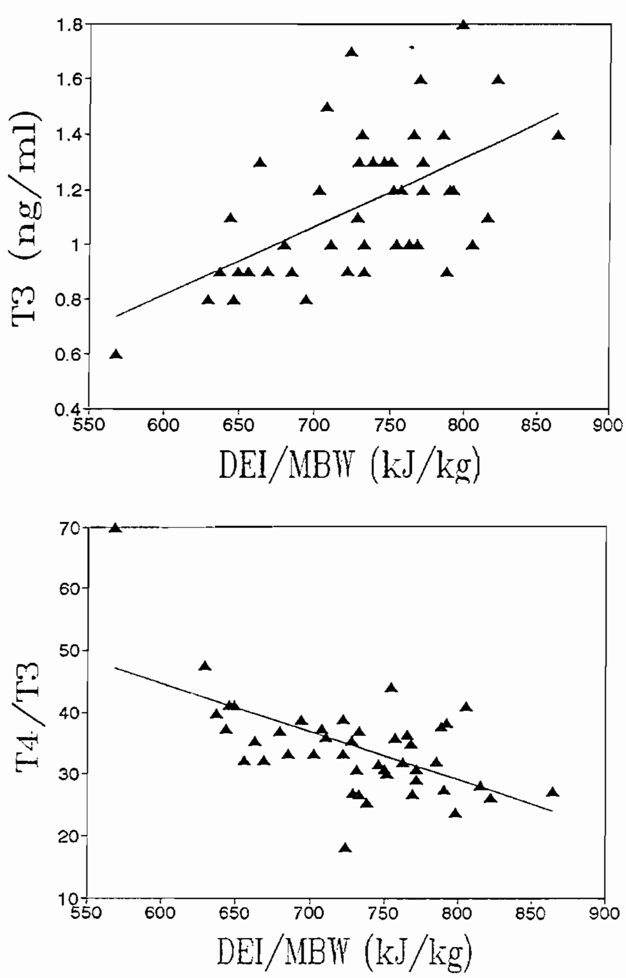

Figure 1. Relationships between T_3, T_4/T_3 ratio and DEI/MBW

T_4 (37.8 vs 38.0 ng/ml) concentrations nor the T_4/T_3 ratio (33.9 vs 35. 1) significantly. T_3 (1.04 vs 1.16 ng/ml) and T_4 (38.7 vs 36.6 ng/ml) plasma levels did not appear to be significantly influenced by heat stress conditions. Only the T_4/T_3 ratio (37.4 vs 31.6. P<0.05) significantly increased.

Table III presents only the significant correlations and the relative linear regression equations between the endocrine profile (T_3 . T_4) and the productive performance, they are graphically represented in figure 1. The DEI/MBW is correlated positively with the plasma T_3 level (r = 0.563, P<0.01) and negatively with the T_4/T_3 ratio (r = -0.589, P<0.01).

Table III. Linear regression equations (1) relating plasma T3 (yl)t T4/T3 (y2) and DEI/MBW (x l)

Linear regression equation	Residual Standard Error	r
yl = - 0.676 +0.002 xl	0.2225	0.563**
v2 = 91.462 - 0.078 xl	6.5425	-0.589**

(1) Number of observations: 44
** = P<0.01

4. Discussion

The absence of interaction between age and rearing temperature indicates a similarity of growth and physiological reactions to heat stress conditions in animals of both ages considered.

As concerns the productive performance in relation to age, the older rabbits exhibited higher final live weight and daily intake with a significant worsening of feed efficiency. The negative effect of increasing age on feed efficiency was already observed in previous experiments (6, 17). A marked effect of the environmental temperature was compared to the younger rabbits, observed on almost all field performances: heat stress induced a significant decrease of final body weight and growth rate because of a lower feed intake, thus decreasing the extra heat to be dissipated to the environment and limiting the energy loss due to thermolysis.

As concerns hormonal profiles, age did not have a significant effect on steroids levels, due to the limited interval of time considered during this stage of life. In fact. Berger *et al.* (2, 3) and Chiericato (5) found a significant increase in plasma T and DHT levels in growing rabbits. considering however a larger time interval. The period of time considered in this experiment enabled us to observe only a significant variation of T/DHT ratio, caused by a decrease of T with respect to an increase of DHT in rabbits aged between 71 and 85 days. As the nutritive level influences the steroid plasma concentrations (4, 5), these results have to be considered in relation to the feeding plan. The more intensive feeding adopted in this experiment led the rabbits to higher precocity levels. reaching sexual maturity earlier, with higher androgen plasma levels. These findings. together with those reported in a previous experimental work (5) point out the importance of feeding program effects and their possible interactions with age on the androgen plasma concentrations in rabbits. Going on to consider the effects of temperature, the androgens exhibited plasma values tendentially lower in the heat stressed animals. Similar results were obtained in a previous study where a wider temperature range than the present (12°C to 30°C) was adopted (4). These results, in view of the indications of a previous research work (4), suggest studying the relationships between nutritive levels. ambient thermal conditions and plasma androgen concentrations, considering a wider range of temperatures. A comparison of these data with those obtained by other authors is difficult, particularly for testosterone because of the different concentrations in relation to age and the well-known pulsatile pattern (15, 19) of this hormone. However, the present values are within the range reported by other experimental contributions carried out on rabbits (1, 4, 5, 15, 19).

The blood sampling carried out on 85 day-old rabbits, compared to that observed at 71 days of age, did not show a significant difference in plasma C levels. but only a tendential decrease. Given the scarcity of available literature on this topic, the decrease of cortisol levels in growing rabbits is hardly documented. Our findings are in agreement with results obtained by other researchers (16) who observed a cortisol decrease in plasma with increasing age (50 d to 80 d). In other monogastric species, such as swine (11, 14) cortisol is affected by the age of the animals. Hot environment conditions were not able to influence the plasma C levels notably. It is possible to point out that the rabbits reached a homeostatic state probably overcoming the initial alarm reaction stage induced by the high temperature. This conclusion is confirmed by data relating to some corticosteroid hormones obtained by Spanish authors (22) and in our previous trial (4), where a markedly wider temperature range was tested. Our data are not in accordance with the findings of Trammel *et al.* (21) who found a decrease in cortisol levels in heat stressed rabbits adopting temperature levels different from ours (16.8 vs 32.2°C) in a more limited period of time (23 d). Considering other monogastric species such as swine, the cortisol plasma levels (18) present an increase in presence of low rearing temperatures due to calorigen action. The cortisol levels of this trial are similar to those found at the University of Arkansas (2 1) and in Italy (4).

Going on to consider the thyroid hormones, they appeared to be similar between the two age categories of rabbits studied. There is a substantial absence of experimental contributions on the effects of age on these hormones in rabbits. In chickens, T_3 concentrations decreased throughout the productive cycle, while T_4 increased with increasing age (12, 13). In relation to temperature effects, only the T_4/T_3 ratio is affected significantly by the treatment, increasing in the rabbits kept at 27°C. This variation is due to an increase in T_4 and to a decrease in T_3 in hot conditions. The results reported by literature are not homogeneous (16, 21) due to the different environmental temperature conditions tested. This fact points out the need for further studies to be conducted in similar environmental conditions. thus enabling comparison. It may be worth mentioning that hormonal response to environmental temperature can be opposite, depending on the length of thermal exposure (7). The temperature effect on thyroid functions has been studied in other farm species, such as poultry. In chickens, T_3 decrement and T_4 increment were observed with increasing temperature (20). Hormonal levels exhibited by the rabbits in this trial are similar to those found by other researchers (8, 16, 21).

The productive performance was significantly correlated with the endocrine response of the animals. In particular, T_3 was positively affected by DEI/MBW. while T_4/T_3 ratio appeared to be influenced negatively by DEI/MBW. It is known that the increase of caloric intake in individuals results in an increased diet-induced thermogenesis; the increased levels of T_3 apparently derive from increased peripheral conversion of T_4 to T_3 and a decreased conversion of T_3 to rT_3 (9). Similar relationships were found in our previous work (4) where a wider range of temperature was adopted, pointing out that the thyroid endocrine response takes place in a rather wide range of thermal values.

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Literature

1. Berger M., Chazaud J.. Jean-Faucher Ch., de Turckheim M., Veyssiere G. & Jean Cl., 1976. Developmental patterns of plasma and testicular testosterone in rabbits from birth to 90 days of age. Biol. Reprod. **15**, 561-564.

2. Berger M., Corre M., Jean-Faucher Ch.. de Turckheim M., Veyssiere G. & Jean Cl.. 1979. Changes in the testosterone to dihydrotestosterone ratio in plasma and testes of maturing rabbits. Endocrinology **5**, 1450-1454.

3. Berger M., Jean-Faucher Ch., de Turckeim M., Veyssiere G., Blanc M. R., Poirier J. C. & Jean Cl., 1982. Testosterone. luteinizing hormone (LH) and follicle stimulating hormone (FSH) in plasma of rabbit from birth to adulthood. Correlation with sexual and behavioural development. Acta Endocrinologica **99**, 459-465.

4. Boiti C., Chiericato G.M., Filotto U. & Canali C.. 1992. Effects of high environmental temperature on plasma testosterone, cortisol, T_3 and T_4 levels in the growing rabbit. Proc. Vth World Rabbit Congress, Oregon State University. Corvallis, Vol. **1**. 447-455.

5. Chiericato G. M., 1984. Influenza del livello nutritivo sulla concentrazione periferica di alcuni ormoni androgeni nel coniglio. Proc. IIIrd World Rabbit Congress, Roma. Vol. **1**. 287-293.

6. Chiericato G. M., Ravarotto L. & Rizzi C., 1992. Effetto dell'età sul profilo metabolico del coniglio in accrescimento di razza Nuova Zelanda Bianca. Atti XLVI Convegno Nazionale S.I.S.Vet., Venezia.

7. Decuypere E. & Kühn E. R., 1988. Alteractions in thyroid hormone physiology induced by temperature and feeding early hatched chickens. Acta Physiol. Pol. **39**, 380-394.

8. Fekete S. &. Rudas P., 1988. Serum thyroid hormone levels and outer-ring monodeiodination of thyroxine in rabbits treated with different amounts of perchloric acid ammonia (PAA). Proc. 4th World Rabbit Congress, Budapest. Vol. **2**. 434-440.

9. Hadley Mac E., 1992. Thyroid hormones. In: Endocrinology. p. 346. Prentice Hall Third Ed. Englewood Cliffs, NJ 07632.

10. Harvey W. R., 1990. LSMLMW. PC 2 Version. Mixed Model Least Squares and Maximum Likelihood Computer Program. Department of Dairy Science, Ohio State University., Columbus, Ohio.

11. Kirkwood R. N., Evans F. D. & Aherne F. X., 1987. Influence of age, weight and growth rate on basal LH, growth hormone and cortisol, and estrogen-induced LH release in prepubertal gilts. Can. J. Anim. Sci. **67**, 1001-1010.

12. Kühn E. R., Decuypere E., Colen L. M. & Michels H., 1982. Posthatch growth and development of a circadian rhythm for thyroid hormones in chicks incubated at different temperatures. Poultry Sci. **64**, 540-549.

13. Leenstra F. R., Decuypere E., Beuving G., Buyse J., Berghman L. & Herremans M., 1991. Concentrations of hormones. glucose, triglycerides and free fatty acids in the plasma of broiler chickens selected for weight gain or food conversion. Br. Poultry Sci. **32**. 619-632.

14. Mc Cauley I. & Hartmann P. E., 1984. Changes in piglet leucocytes. B limphocytes and plasma cortisol from birth to three weeks after weaning. Research in Veterinary Science **37**, 234-241

15. Moor B. C. & Younglai E. V., 1975. Variations in peripheral levels of LH and testosterone in adult male rabbits. J. Reprod. Fert. **42**. 259-266.

16. Morera P., Kuzminsky G. & Finzi A., 1991. Effetto della manipolazione su alcuni parametri fisiologici del coniglio sottoposto a stress termico. Coniglicoltura **28**, 3 9-4 1.

17. Parigi-Bini R., Xiccato G., Cinetto M., Dalle Zotte A. & Converso R., 1992. Effetto dell'età del peso di macellazione e del sesso sulla qualità della carcassa e della carne cunicola I. Rilievi di macellazione e qualità della carcassa. Zoot. Nutr. Anim. **18**, 157-172.

18. Rafai R, Neumann R., Leonhardt W., Frenyo L., Rudas P., Fodor L. & Boros G., 1988. Effect of environmental temperature on pigs infected with Pasteurella multocida type A. Pig News and Inf. 9, 830 (abstr).

19. Rowe P. H., Hopkinson C. N. R., Shenton J. C. & Glover T. D., 1976. The secretion of LH and testosterone in the rabbit. Steroids **25**, 313-322.

20. Sinurat A. P., Balnave D. & Mc Dowell G. H., 1987. Growth performance and concentrations of thyroid hormones and growth hormone in plasma of broilers at high temperatures. Aus. J. Biol. Sci. **40**, 443-450.

21. Trammel T.L., Stallcup O. T., Harris G. C., Daniels L. B. & Rakes J. M., 1989. Effects of high temperature on certain blood hormones and metabolites and on reproduction in rabbit does. J. Appl. Rabbit Res. **12**, 101-102.

22. Verde M. P. & Piquer J. G., 1986. Effect of stress on the corticosterone and ascorbic acid (vitamin C) content of the blood plasma of rabbits. J. Appl. Rabbit Res. **9**, 181-185

G. M. Chiericato: Italian, Full Professor of Animal Science
C. Boiti: Italian, Full Professor of Animal Physiology
C. Rizzi: Italian, PhD in Animal Science
C. Canali: Italian, researcher in Animal Physiology
Vania Rostellato: Italian, she is beginning PhD of Animal Science

PRESS RELEASE

SECOND INTERNATIONAL WORKSHOP ON THE IMPROVEMENT OF AFRICAN PEAR AND OTHER NEW SOURCES OF VEGETABLES OILS

The second international workshop on the improvement of African Pear and other new sources of vegetable oils took place at Ngaoundéré from 3rd to 5th December 1997 in the premises of the Advanced School of Agro-process Industries (ENSAI) of Ngaoundéré University (Cameroon). This workshop was sponsored by the African Training for Leadership and Advanced Skill (ATLAS). African-American Institute (AAI), New York, USA, International Service of Appropriate Technology, GTZ, Germany, The (CTA), ACP-Lomé Convention and the International Commission of Agricultural Engineering (CIGR), Belgium.

The agenda included opening ceremony, samples show, presentations, panel discussions, meeting of the scientitic organisation (ASANET, ACAGER), visit of the factory and African pear accessions, and closing ceremony.

The number of participants at this workshop was 74 with 64 scholars/professionnals., and 10 academic staff. The number of women was 11. Among participants, 14 came from abroad with 11 from Africa : Ivory-coast (3), Gabon (1), Mali (1), Niger (1), Nigeria (4), South Africa (1) and 3 from Europe : France (2) and Germany (1), and 60 from Cameroon with 35 from Ngaoundéré.

Among 64 scholars/professionnals, the distribution was as follows:
- 89.5% of scholars
- 4.5% of non governmental organisation delegates
- 3.0% of industrials
- 3.0% sponsor representatives

For any information concerning the workshop proceedings
Please contact : Dr. C. KAPSEU, Coordinator
2nd Workshop on african pear and other vegetable oils
ENSAMUT, University of Ngaoundéré
P. 0. Box 455 Ngaoundéré, CAMEROON
Tel./Fax: (237) 25 27 51