F. TOTEDA¹,
A. FACCIOLONGO², M.RAGNI¹,
A. VICENTI¹

Effect of suckling type and PUFA use on productive performances, quantiqualitative characteristics of meat and fatty acid profile in lamb

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TITOLO
Influenza del tipo di
allattamento e dell'uso dei
PUFA sulle performance
produttive, sulle caratteristiche
quanti-qualitative e sul profilo
acidico della carne di agnello

KEY WORDS Lamb, suckling, PUFA, meat quality, fatty acids

PAROLE CHIAVE Agnelli, allattamento, PUFA, qualità della carne, acidi grassi

¹Dipartimento di Produzione Animale, Università degli Studi di Bari "Aldo Moro", Italy ²CNR, Istituto di Genetica Vegetale, Bari, Italy

Indirizzo per la corrispondenza:
Prof. Francesco Toteda
Dipartimento di Produzione Animale
Università degli Studi di Bari "Aldo Moro "
Via G. Amendola, 165/A 70126, Bari
Tel. +39 080 5442836
Fax. +39 080 5442822
E-mail: toteda@agr.uniba.it

Summary

In March, 24 male lambs were divided into three groups and fed as follows: maternal milk (MM); acidified milk replacer (MR); acidified milk replacer with 5% of the fat total amount replaced by a linseed and fish oil mixture (MR+PUFA). Milk consumption and live weight were recorded weekly. At slaughter we recorded the carcass composition, pH, tenderness, colorimetric characteristics, chemical and fatty acid composition of the meat. Natural suckling resulted in a better slaughter yield (P < 0.01), fatter carcasses, and meat which was more tender (P < 0.01), less bright (P < 0.01) and redder (P < 0.05) than the two experimental groups. Meat from naturally suckled lambs contained higher (P < 0.01) concentrations of saturated fatty acids (SFA) than the other groups. Samples from the MR+PUFA group showed higher (P < 0.01) levels of PUFA and a better PUFA/SFA ratio. The groups reared artificially showed atherogenicity and the thrombogenicity index lower (P < 0.01) than the group naturally feeding.

Riassunto

Nel mese di marzo, 24 agnelli sono stati divisi in tre gruppi e alimentati come segue: a) latte materno (MM); b) sostituto del latte acidificato (MR); c) sostituto del latte acidificato con sostituzione del 5% dei lipidi totali con una miscela di olio di lino e di pesce ricca in PUFA (MR + PUFA). L'allattamento naturale condiziona una migliore resa alla macellazione (P < 0.01), carcasse più grasse e carne significativamente (P < 0.01) più tenera (P < 0.01), meno luminosa e più rossa (P < 0.05). La carne degli agnelli allattati naturalmente evidenzia una maggiore concentrazione di acidi grassi saturi (SFA) mentre quella dei soggetti appartenenti al gruppo MR+PUFA risulta più ricca (P < 0.01) di acidi grassi polinsaturi (PUFA) e presenta un rapporto PUFA/SFA significativamente più elevato. L'allattamento artificiale ha determinato indici di aterogenicità e di trombogenicità marcatamente più bassi (P < 0.01).

Introduction

Scientific evidence has shown that a diet without animal fats and with high levels of unsaturated (UFA) and polyunsaturated fatty acids (PUFA) not only reduces the incidence of cardiovascular disease in humans, but also has beneficial effects on the immune system and on the prevention of some cancers (1, 2). Saturated fatty acids (SFA) are considered as risk factors for coronary disease and - with the exception of stearic acid (3) - tend to increase total plasma cholesterol levels and LDL-conveyed cholesterol, while unsaturated fatty acids lower these levels (4, 5).

The n-6 and n-3 PUFA series are important in nutritional terms because some of them are essential, and so are available exclusively from food (6). Recent research has emphasized the biological importance of n-3 fatty acids, especially EPA (C20:5n-3) and DHA (C22:6 n-3) (7) because they play a key role in development and function of the brain and retina (8).

Meat is an important component of the human diet in industrialized countries, and so the livestock sector has paid special attention to quality in terms of lipid fraction improvement. Feed formulation is a very decisive strategy used to increase the concentration of muscle PUFA in ruminants. The influence of rations supplemented with

fish oil or vegetable oils (coconut, olive, sunflower, canola, etc..) given to lambs (9-12) and kids (13) has been documented. It should be noted that the fat tissue composition of monogastric and preruminant animals reflects that of diet-ingested fat (14, 15) while, in ruminants, unsaturated fatty acids contained in the ration are subjected to ruminal bio-hydrogenation processes (11, 15, 16). For this reason, the fatty acid constituents of the muscles and tissues are very different from those in the diet. Lambs are fed traditionally only on maternal milk, although many farms successfully use artificial suckling (17). Although artificial rearing can have detrimental effects on lamb welfare (18), this technique has a number of advantages: profitability when maternal milk is marketed; adequate feeding in case of twin births or orphaned animals (19); increased age and lamb weight at slaughter; and protection of lambs from maternal diseases (20). However, results about the influence of artificial suckling on productive performance and carcass quanti-qualitative characteristics are contradictory (21-23). With this background, our study has investigated how the milk type and the addition of polyunsaturated fatty acids to milk replacers influences the productive performances and the quantitative and

qualitative characteristics of Comisana lambs meat.

Material and methods

Experimental design and animal management

The research was carried out at a farm in the Province of Bari (Italy) (41° N, 400 m above sea-level) in accordance with the animal welfare regulations (91/629/EEC Directive, acknowledged in Italy by D. Lgs. 533/92 and modified by D. Lgs. 331/98). In March, 24 twin male "Comisana" lambs were divided into three homogeneous groups (n = 8) and given the following feed treatments: maternal milk (MM group); acidified milk replacer (MR group); acidified milk replacer with 5% of the fat total amount replaced by a linseed and fish oil mixture (MR + PUFA group). Lambs in the MR and MR+PUFA groups were separated from their dams two days after birth.

Artificial rearing took place in single 0.80 m² boxes in a closed ventilated barn, where the average temperature during the trial ranged between 15 and 20°C. Bedding was covered with a plastic net in order to avoid ingestion of litter.

The milk replacer was prepared with 200 g milk powder/litre water, kept at room temperature and distributed 3 times daily in buck-

ets equipped with teats from which lambs could suck the milk as required.

The malted milk had the following percentage composition: moisture 3.0, crude protein 24.0, lipids rough, 25.0, raw cellulose, 0.3 and ash 7.0. The fatty acid composition of the acidified milk replacer and ewes' milk (samples taken every 10 days) is given in table 1. Milk consumption was recorded weekly after fat percentage standardization to 6.5% (5.43% for natural and 4.1% for artificial milk) (24), and live weights were also recorded weekly, in order to calculate the daily weight gain and the feed conversion index. To evaluate milk consumption by natural suckling, lambs were individually weighed, weekly, before and after each feed. Due to health problems, one subject was discarded from each of the MR and MR+PUFA groups for the statistical processing of data.

At the end of the experimental period (5 weeks) the lambs were slaughtered (according to veterinary police rules: D.P.R.320/54) after fasting for 12 hours. Carcass data collected included hot carcass weight (HCW), cold carcass weight (CCW, chilled 24 h); dressing percentage was calculated as HCW/fasted live weight x 100. The carcasses were divided into two symmetrical halves and refrigerated for 24 hours at 4°C before

being sectioned into cuts. The pelvic limb and the lumbar region were dissected into tissue components (lean, fat and bone).

Physical analysis

The pH values of the *Longissimus lumborum* (*Ll*) muscle were measured at slaughtering (pH₀), and after 24 hours (pH₂₄) of cooling. Afterwards representative sub-samples were taken from the same muscle and analyzed for: a) meat colorimetric characteristics; and b) shear force. The colorimetric indices (L* = Lightness; a* = redness index; b* = yellowness index) were

assessed using a Hunter Lab MiniScan™ XE Spectrophotometer (Model 4500/L, 45/0LAV, 3.20 cm diameter aperture, 10° standard observer, focusing at 25 mm, illuminant D65/10; Hunter Associates Laboratory, Inc.; Reston, Virginia, USA) and by taking three readings for each sample. Shear force was determined according to the Warner Bratzler Shear (WBS) system using an Instron 5544.

Chemical analysis

Raw meat samples (100 g) devoid of external fat from the *Ll* muscle

Table 1 - Fatty acidic composition (%) of ewes' milk and of acidified milk replacer diets with or without PUFA supplement

Fatty acid	Ewes' milk	Acidified milk replacer	Acidified milk replacer + PUFA ⁽¹⁾
C12:0	4.20	10.30	7.70
C14:0	9.90	6.50	5.40
C16:0	21.00	22.10	20.80
C18:0	7.60	16.00	13.80
C16:1n-7	1.30	2.20	2.10
C18:1n-7	0.60	1.10	1.10
C18:1n-9	19.00	31.0	30.50
C18:2n-6	2.50	3.80	5.30
C18:3n-6	0.10	0.40	1.10
C18:3n-3	0.50	0.20	1.60
C20:3n-6	0.20	0.10	0.20
C20:3n-3	0.10	0.00	0.10
C20:4n-6	0.30	0.00	0.30
C20:5n-3	0.10	0.00	0.50
C22:6n-3	0.00	0.00	2.10

^{(1) 5%} of the fat total amount replaced by a linseed and fish oil mixture

were used to analyze the chemical composition and fatty acid profile (% of total fatty acids). The chemical composition was determined using samples cut into cubes and homogenized using a double rotating blade homogenizer. Lipids were extracted according to the Folch et al. (25) method with a 2:1 chloroform/methanol solution (v/v). The fatty acids were methylated using a BF3/methanol solution (12% v/v) (26) and analyzed using a gas chromatograph (Chromopack CP 9000) with a silicated glass capillary column (70% Cyanopropyl polysilphenylenesiloxane BPX 70 of SGE Analytical Science; length = 50 m; internal diameter = 0.22 mm; film thickness = 0.25 µm; temperature program = 135°C for 7 minutes; temperature increase = 4°C up to 210°C). The same method was also applied to milk samples.

The meat risk factor was estimated using the atherogenicity (AI) and thrombogenicity (TI) indices (27) and by nutritional value calculation (stearic acid + oleic acid/palmitic acid) (3).

Statistical analysis

Statistical analyses was performed using the GLM procedure of the SAS application package; means were compared using Student's t test (28).

Results and discussion

Productive performances

MM lambs showed daily average weight gains, daily milk consumption and food conversion indices which were not statistically different when compared with those of MR group (Tab. 2). However, the n-3 replacement (MR+PUFA) had a markedly negative effect (P < 0.05) on daily weight gain (194 g/d) and on the food conversion index (5.90 kg/kg). The daily weight gains of MM and MR groups were similar to the values recorded in some research where

artificial suckling does not affect weight gains (20, 21), but contrast with the results of other authors who adopted this rearing system and found that performances worsened (22) or improved (17). The milk conversion indices, also, present values which are in agreement with other experimental data (29) or indicate a more satisfactory food conversion capacity (30). The difference in genotype, the quantity and quality of maternal milk, the chemical composition and/or method of milk distribution could probably account for the differences between the results.

Table 2 - *In vivo* performances and slaughtering data (% of empty body weight)

Variable	Group			$\mathrm{SD}^{\scriptscriptstyle{(2)}}$
	MM	MR	MR+PUFA	DF = 19
Initial live weight (kg)	5.501	5.218	5.225	0.740
Weight gain (g/d)	229ª	$228^{\rm ab}$	194 ^b	41.673
Milk consumption (1) (g/d)	1208	1291	1134	275.355
Feed conversion index (kg/kg)	5.08 ^b	5.62ab	5.90 ^a	0.678
Empty body weight (kg)	13.16	12.76	11.47	1.550
Hide	11.00	10.02	10.98	1.688
Empty digestive tract	8.91	11.00	10.93	2.484
Omentum	0.77	0.55	0.47	0.298
Head	6.09	5.74	6.24	0.884
Pluck	4.82Bb	5.37 ^{AB}	5.57 ^A	0.439
Shanks	0.88	0.83	0.93	0.111
Net warm dressing percentage	73.60^{A}	66.31 ^B	65.29 ^B	1.918
Chilling loss	4.04^{a}	2.72 ^b	$3.00^{\rm b}$	0.883

⁽¹⁾ Standardized at 6.5% of fat. (2) SD = standard deviation of the mean;

a, b: P < 0.05; A,B: P < 0.01

Slaughtering data

Natural suckling resulted in a slightly higher value of empty body weight and of omentum, and hide proportion (Tab. 2). Only the incidence of pluck was markedly lower in MM than MR (P < 0.05) and MR + PUFA (P < 0.01) groups.

The net warm dressing percentage was similar in the MR and MR + PUFA groups, and significantly (P < 0.01) higher in MM group than the two experimental groups. The positive influence of natural rearing recorded in our research is consistent with data reported by Vergara and Gallego (23), but contrasts with lower results reported by Girolami et al. (31) and Napolitano et al. (22) and with results reported by other authors who have found similar slaughtering yields for the two feeding systems (17, 21, 32); this contrasting trend may be due to different genotypes and/or to different slaughtering weight (33).

The chilling loss of carcasses was significantly (P < 0.05) higher for MM group than the others groups and is in agreement with observations made on Apulian lambs (17).

Section and dissection data

The carcasses of MM group (Tab. 3) are heavier, although this was

Table 3 - Sectioning (% of carcass weight) and dissecting data

Parameters	Group			SD
	MM	MR	MR+PUFA	DF = 19
Carcass weight (kg)	7.14 ^A	6.47 ^{AB}	5.54 ^B	0.924
Neck	7.16 ^b	8.38^{a}	8.40^{a}	0.952
Steaks + Brisket	24.62	26.01	24.92	2.445
Shoulder	19.81ª	18.34^{b}	19.01ab	1.009
Loin	8.08	9.10	8.46	1.634
Abdominal region	3.48	3.70	3.35	0.961
Leg	34.09	32.14	33.64	2.119
Kidney fat	1.52ª	1.20ab	0.96^{b}	0.441
Kidneys	1.22	1.11	1.25	0.141
Leg (Kg)	2.424^{Aa}	2.046^{AB}	1.837 ^{Bb}	0.274
Lean (%)	62.82	63.99	62.41	3.244
Fat (%)	9.17^{a}	7.60ab	$5.80^{\rm b}$	2.715
Bone %	28.00^{5}	28.42 ^b	31.78ª	3.019
Lean/bone	2.27	2.28	1.99	0.315
Lean + fat/bone	2.60^{a}	2.55ab	2.18 ^b	0.364
Lean/fat	8.11 ^b	8.84ab	11.72°	3.265
Loin (kg)	0.627^{a}	0.568^{ab}	0.463 ^b	0.134
Lean (%)	53.20	50.00	54.67	5.045
Fat %	18.16ª	15.42ab	$11.00^{\rm b}$	5.810
Bone %	28.64Bb	34.57^{Aa}	34.31^{ABa}	3.779
Lean/bone	1.90^{A}	1.45^{B}	1.61^{AB}	0.274
Lean + fat/bone	2.56^{A}	1.91^{B}	$1.94^{\rm B}$	0.377
Lean/fat	3.15 ^b	4.38ab	5.03ª	1.611

a,b: P < 0.05; A,B: P < 0.01

statistically significant (P < 0.01) only in comparison with MR+PUFA group. MM lambs showed a significantly (P < 0.05) lower neck percentage than MR and MR+PUFA lambs, and a higher shoulder percentage than subject from MR group, and higher kidney fat percentage than those from MR+ PUFA groups. The incidence of shoulder and

neck is similar to that reported for lambs of similar weight (34, 35). The dissection data indicated that there were no marked differences between the groups for the lean incidence in the loin and leg cuts (Tab. 3); however, the levels recorded for the leg are higher than those reported by Perez et al. (34) in Suffolk lambs, but similar to those recorded by Ruiz de Hi-

dobro and Caneque (36). These discrepancies could be justified by the muscle growth rate, which in different breeds may also be dissimilar.

However, MM group had significantly greater (P < 0.05) values for the fat percentages than the MR+PUFA group, both for leg and for loin. MR+PUFA group also had a markedly (P < 0.05) higher incidence of bone in the leg than the other two groups while the loin bone incidence of MM group was significantly lower than MR (P < 0.01) and MR + PUFA (P < 0.05) groups. In the MR+PUFA group, the lower fat incidence and higher bone incidence could be due to lower carcass weight as observed in Suffolk Down lambs (34). MR+PUFA group had a not significant value of lean/bone ratio for the leg, while lean + fat/bone ratios appeared significantly (P < 0.05) lower and lean/fat ratios resulted significantly (P < 0.05) higher than MM group. The lean/bone and lean+ fat/bone ratios observed are similar to other research (34) while the lean/fat ratio is similar to what we reported earlier (17), although much higher than that observed in Suffolk lambs (34) and Manchega lambs (36).

The lean/bone ratio of the loin was also higher in MM lambs, although the difference was statistically significant (P < 0.01) only in

comparison with the MR group. The lean+fat/bone ratio of MM lambs was significantly (P < 0.01) higher compared to both artificially-fed groups, whereas the lean/fat quotient was smaller and markedly (P < 0.05) different from MR+PUFA group.

The higher levels of fat in MM group, as shown by omentum, kidney fat, leg and loin fat values, is probably due to higher carcass weight (34). Some researchers report that a high growth rate translates into greater development of adipose tissue (37, 38) and others record fattier carcasses in lambs fed *ad libitum* (39).

Chemical and physical characteristics of meat

The meat chemical composition was not influenced by diet (Tab. 4) and it is similar to meat of the Appennine (40) and the Assaf lambs (41)

There were no significant differences in pH₀ between dietary

treatments (Tab. 5); values ranged between 6.60 and 6.69, in agreement with other research (42, 43). Moreover, values consistent with ours, and without differences attributable to the type of feeding (21), were observed in Sardinian lambs reared in the South of Italy and slaughtered at the same age. After 24 hours the pH fell by 0.66-0.75 points, but remained within the normal range necessary to prevent meat color alterations (44). The pH₂₄ values are comparable to results from other breeds reared in the Mediterranean area (21, 23, 45,) and higher than those reported by other authors about breeds reared in different geographical areas (46).

Table 5 reports the colorimetric characteristics of the meat. Natural suckling determined significantly (P < 0.01) lower brightness (L *) and yellowness (b *) indices than artificial feeding; the MM group had a significantly (P < 0.05) higher level of redness (a*) only in comparison with the MR

Table 4 - Meat chemical composition (% on as it is basis)

	MM	Group MR	MR+PUFA	SD DF = 19
Moisture	73.57	73.93	74.44	0.879
Protein	20.03	20.05	19.70	0.855
Fat	3.84	3.66	3.24	0.677
Ash	1.22	1.29	1.26	0.074

Table 5 - pH, colorimetric characteristics and tenderness of *Longissimus lum-borum* muscle

Parameters		Group		SD
	MM	MR	MR+PUFA	DF = 19
pH_0	6.60	6.63	6.69	0.166
pH_{24}	5.94	5.87	5.96	0.133
$\Delta_{\text{0-24}}$ pH	0.66	0.75	0.73	0.213
L^*	37.23 ^B	44.88^{A}	43.73 ^A	2.493
a*	8.63ª	7.83 ^b	8.21 ^{ab}	0.738
b*	6.64 ^B	11.13 ^A	10.71 ^A	1.309
WBS (Kg/cm ²)	1.68 ^B	2.48 ^A	3.00^{A}	0.562

a,b: P < 0.05; A,B: P < 0.01

group. These data contrast both with those recorded earlier in Apulian lambs (17), which showed similar L* and b* values for the different types of suckling, and also contrasts with other authors who observed a greater brightness index in the meat of naturallysuckled lambs (23, 32, 38). The differences can probably be attributed to the different genotypes used (45), to variations in the duration and temperature of meat storage (47) and also to a different content of intramuscular fat (23, 48).

The meat of MM lambs (Tab. 5) was significantly (P < 0.01) more tender than MR and MR+PUFA groups, this is probably due to its higher fat content (49).

Fatty acid composition of meat

The fatty acid composition of lipid tissue was generally influ-

enced by feed ingested, in agreement with what has been observed in lambs (32, 50) and kids (51). Oleic acid (C18:1n-9) was present in the greatest quantity, followed by palmitic (C16:0) and stearic (C18:0) acid, as observed in other studies (50, 52).

The saturated fatty acids (SFA) were significantly (P < 0.01) higher in naturally-fed lambs; C18:0 acid was the only one which showed a significantly (P < 0.05) higher level for the artificially-suckled lambs (Tab. 6). Artificial suckling caused a significantly higher level of monounsaturated fatty acids (MUFA) than a diet of natural milk. In this case there were significantly higher levels of MUFA in MR group fat than in both MM (P < 0.01) or MR + PUFA (P < 0.05) groups.

The polyunsaturated fatty acids (PUFA) were found at significantly (P < 0.01) higher levels in the

MR+PUFA group than the other two groups.

In our study, the PUFA/SFA ratio of MM meat was 0.23 (Table 6), and this is higher than observed in other research (50, 53). This result can be attributed to different genotypes, as shown by comparative studies carried out on sheep (54) and goats (55). The fatty acid composition in adipose deposits can also be influenced by diet, sex and slaughtering age (56). However, in the MR+PUFA group, this ratio is significantly (P < 0.01) higher and is similar to the ratio recommended (0.45) for humans by the Department of Health (57). Particular attention is necessary that the n-6/n-3 ratio does not exceed 4 (57) because this represents a risk factor for cancer and coronary diseases (58). In our study, this ratio ranged between 3.35 and 3.95 and was not influenced by the feeding treatment (Tab. 6).

In general, health indices (atherogenicity and thrombogenicity) were significantly and negatively influenced by natural suckling (Tab. 6).

The nutritional value (stearic acid + oleic acid/palmitic acid) indicates the healthiness of diet in relation to its fat content. Palmitic acid (C16:0) tends to increase blood cholesterol, while stearic (C18: 0) acid has no effect on it, and oleic acid (C18:1) decreases it (3). This parameter should fall be-

Table 6 - Fatty acids composition (% of total fatty acids) and health indices of *Longissimus lumborum* muscle

Fatty acid	Group			SD
•	MM	MR	MR+PUFA	DF = 19
C12:0	1.04	0.43	0.42	0.057
C14:0	6.93 ^A	3.15^{B}	3.25^{B}	0.711
C16:0	21.69^{A}	17.10^{B}	16.25 ^B	2.889
C16:1n-7	2.51^{Bc}	3.40^{Aa}	2.88^{ABb}	0.090
C18:0	11.04 ^b	12.90^{a}	12.73ª	1.938
C18:1n-9	35.66^{Bc}	46.35^{Aa}	40.53^{ABb}	14.079
C18:2n-6	5.80^{B}	6.40^{B}	9.57^{A}	1.970
C18:3n-6	0.34	0.20	0.32	0.132
C18:3n-3	0.11	0.10	0.12	0.003
C20:4n-6	0.21	0.30	0.38	0.038
C20:5n-3	1.01 ^b	1.38ab	1.85ª	0.328
C22:5n-3	0.16	0.00	0.05	0.028
C22:6n-3	0.06	0.00	0.08	0.007
∑ SFA	42.83 ^A	34.53 ^B	34.13 ^B	10.215
∑ MUFA	47.51 ^B	55.93^{Aa}	50.97^{ABb}	10.183
∑ PUFA	9.66^{B}	9.55^{B}	14.88^{A}	8.972
∑ n-6 fatty acids	6.66^{B}	7.25^{B}	10.98^{A}	2.092
\sum n-3 fatty acids	1.77^{B}	1.88^{ABb}	3.48^{Aa}	0.996
n-6/n-3 ratio	3.76	3.95	3.35	1.134
Nutritional value	2.16^{B}	3.47^{A}	3.30^{A}	0.271
PUFA/SFA ratio	0.23^{B}	0.28^{ABa}	0.44^{Ab}	0.110
Atherogenicity index	0.87^{A}	$0.47^{\rm B}$	0.46^{B}	0.114
Thrombogenicity index	1.09^{A}	0.81^{B}	0.73 ^B	0.135

a, b: P < 0.05; A, B: P < 0.01

tween 2 and 3 for meat (16, 59). In our research, the fat nutritional value was 2.16 for MM group and was greatly (P < 0.01) improved by artificial suckling (Table 6).

Conclusions

Bottle-feeding does not affect the productive performances of lambs but has a negative effect on yield at slaughter. The meat pH is not affected by suckling type, while the meat produced by naturally fed lambs shows a lower brightness index (L*) and higher yellow (b*) and red (a*) indices, and it is also more tender. PUFA addition to the diet improved lipid health indices of meat.

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