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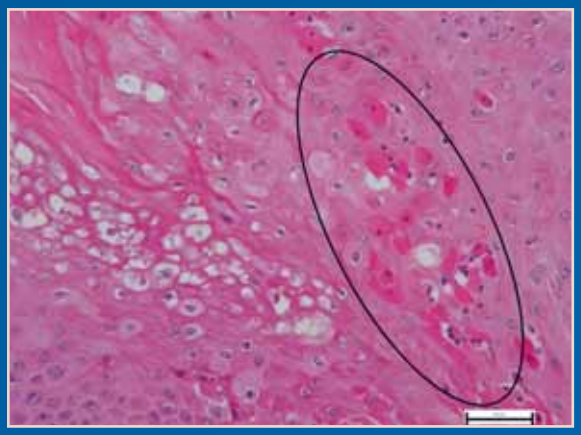

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Stima dell'effetto pascolo sul profilo in acidi grassi del latte bovino in allevamenti di montagna

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RIASSUNTO

La composizione in acidi grassi del latte è stata oggetto negli ultimi decenni di numerosi studi, sia per aspetti tecnologici e organolettici dei prodotti derivati, sia per quelli nutrizionali e di salubrità. La quantità, così come la composizione lipidica del latte, sono in parte caratteri ereditari, ma in gran parte sono determinati dall'alimentazione degli animali. Lo scopo del presente lavoro è quello di caratterizzare il contenuto in acidi grassi saturi ed insaturi del latte bovino prodotto in 37 allevamenti tipici per alimentazione e stabulazione di un'area di montagna del nord Italia (Valle Camonica), tramite una tecnica analitica di stima indiretta basata sulla spettroscopia in infrarosso. Attraverso l'analisi di 189 campioni di latte di massa raccolti tra la primavera e l'estate 2015, si è potuto verificare che il periodo di pascolo influisce in misura statisticamente significativa sul rapporto tra acidi grassi insaturi e saturi del latte: 0,42 (DS 0,025) rapporto medio nelle stalle a fondovalle *vs* 0,57 (DS 0,048) rapporto medio delle stesse mandrie al pascolo. Il presente lavoro fornisce inoltre un quadro complessivo delle caratteristiche del latte di montagna evidenziando alcune peculiarità indotte dal periodo di pascolo. Considerato che la tecnica analitica di screening utilizzata, seppur con un'accuratezza relativamente limitata, garantisce analisi semplici, rapide, a costo molto contenuto ed eseguibili su elevati numeri di campioni anche per periodi prolungati, riteniamo che questo tipo di attività possa concorrere a supportare la produzione di latte e derivati nelle aree di montagna, valorizzandone le componenti di genuinità e salubrità.

PAROLE CHIAVE

Latte, acidi grassi, FTIR, pascolo.

INTRODUZIONE

La componente lipidica del latte bovino è notoriamente la componente più variabile; la sua quantità dipende infatti da numerosi fattori tra i quali i principali sono: razza, alimentazione, stadio di lattazione, numero di lattazioni, condizioni climatiche e modalità di mungitura. Ma anche la composizione stessa del grasso del latte presenta al suo interno ampi margini di variabilità, in parte collegati alla genetica degli animali[1] ed in gran parte all'apporto alimentare ed al successivo metabolismo ruminale[2,3]. Circa il 98% del grasso del latte bovino è costituito da trigliceridi (il rimanente è costituito da fosfolipidi, mono e digliceridi, acidi grassi liberi lineari e ramificati); le tre molecole di acidi grassi, legate alla base fissa di glicerolo, costituiscono la componente variabile che influenza sia gli aspetti nutrizionali e di digeribilità del latte e dei prodotti derivati, sia il comportamento tecnologico nel corso del processo di caseificazione. La composizione in acidi grassi è inoltre considerata uno degli elementi fondamentali per definire la salubrità degli alimenti: si vedano a tal proposito le raccomandazioni FAO e WHO sull'assunzione giornaliera di acidi grassi saturi (in particolare acido miristico e palmitico) o sul rapporto tra alcuni acidi grassi *trans* ed i livelli di colesterolemia[4,5] o, ancora, sull'effetto dell'apporto dietetico di acido linoleico[6]. I latticini sono considerati in genere una rilevante fonte di acidi grassi nella dieta per una parte consistente della popolazione mondiale; da ciò deriva l'interesse riservato allo studio della composizione in acidi grassi del latte in relazione a differenti modalità di allevamento e alimentazione delle bovine. Già segnalati, ad esempio, il significativo effetto del pascolo sul rapporto tra acidi grassi ω6 ed ω3, sulla quantità di insaturi o sulla riduzione dei saturi a lunga-media catena e sulla quantità di acido linoleico coniugato (CLA)[7,8]. Il presente lavoro è finalizzato alla caratterizzazione chimica e tecnologica del latte raccolto in allevamenti bovini tipici di un'area di montagna nel nord Italia (Valle Camonica), con particolare riferimento alla composizione in acidi grassi, alla stima del rapporto tra la frazione satura rispetto a quella insatura ed al confronto tra il periodo di pascolo in alta quota rispetto a quello di stabulazione a fondo valle.

MATERIALI E METODI

Tramite una scheda di caratterizzazione degli allevamenti di bovine della zona, realizzata con la collaborazione del personale addetto all'assistenza tecnica sul territorio (Gruppo Azione Locale Valle Camonica e Val di Scalve), sono stati selezionati 37 allevamenti che per ubicazione, caratteristiche e modalità di allevamento fossero rappresentativi degli "allevamenti di montagna" e quindi ben differenziabili dal tipico allevamento intensivo predominante nell'area di pianura della stessa regione (Lombardia). Gli allevamenti selezionati erano, complessivamente, di piccole dimensioni (media bovine in lattazione 11,8) con prevalenza di vacche razza Bruna Alpina (65%), con quote minoritarie di Pezzata Rossa

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(16%) e Meticcia (16%) e livello produttivo medio-basso (16 litri/capo/die), alimentazione prevalente a fieno di produzione locale con integrazione di concentrati di cereali inferiore al 30% della razione (2-7 kg/capo/die) ed assenza di foraggi insilati (mais o altro). Tra gli elementi fondamentali della selezione degli allevamenti è stato considerato lo spostamento degli animali in pascoli di alta quota nel periodo estivo. In ogni allevamento, ed in ognuna delle 19 "malghe" in cui le bovine erano trasferite per il pascolo, è stato eseguito almeno un campione al mese di latte di massa aziendale, sia nel periodo di stabulazione a fondo valle (Marzo-Maggio 2015, 105 campioni "M") che nel periodo di pascolo (Giugno-Agosto 2015, 84 campioni "AP"). La fase di pascolo in quota era organizzata in 19 differenti raggruppamenti di animali (malghe): in alcune di esse accedevano anche animali provenienti da più di un allevamento tra quelli controllati nella prima fase (malghe incluse nello studio); in altri casi alla malga accedevano invece anche animali provenienti da allevamenti intensivi dell'area di pianura (malghe escluse dallo studio). Infine, soltanto in 6 malghe si è mantenuta la perfetta identità di bovine presenti in 6 allevamenti di fondovalle del primo periodo (malghe incluse nello studio).

Su ciascun campione, addizionato di Sodio Azide come conservante, refrigerato fino alla consegna in laboratorio, si sono realizzate le seguenti determinazioni analitiche entro 48 ore dal prelievo:

– Composizione in grasso, proteine, lattosio, caseine, urea, acidi grassi saturi (SFA), insaturi (UFA), mono e polinsaturi (MUFA e PUFA), con tecnica spettroscopica in medio infrarosso con derivata di Fourier (FTIR, Milcoskan FT 6000, Foss, DK);
– Carica batterica totale con tecnica optofluorometrica con cella di flusso (Bactoscan FC, Foss, DK);
– Cellule somatiche con tecnica optofluorometrica con cella di flusso (Fossomatic FC, Foss, DK);
– Acidità titolabile, secondo la metodica Soxhlet-Henkel.

L'analisi statistica dei risultati è stata realizzata con fogli di calcolo Excel Microsoft.

Sono stati impiegati gli Strumenti di Analisi e/o le formule implementate, e gli strumenti di valutazione grafica disponibili nei fogli di calcolo. Le principali analisi statistiche condotte hanno riguardato: la stima di statistiche parametriche sulla base dati (media, media geometrica, deviazione standard) ed il test di significatività per la differenza fra medie di popolazioni (test T di Student).

Nota: riteniamo importante sottolineare che la tecnica utilizzata per la determinazione delle diverse frazioni di acidi grassi rappresenta una metodica di screening indiretto rispetto a metodiche di riferimento in gas-cromatografia finalizzate a quantificare i singoli acidi grassi (ISO 15885:2002[9]); alla validazione di base fornita dal produttore deve di conseguenza essere sovrapposta una calibrazione realizzata dal singolo laboratorio[10] stimata dal confronto con metodiche di riferimento su campioni rappresentativi del latte normalmente analizzato. Ciò determina, che a fronte di un'elevata ripetibilità, l'accuratezza della stima risulta relativamente limitata: ripetibilità (r=2,83 *Sr) compresa tra 0,03 e 0,10 g/100 g ed Errore Standard di stima tra 0,04 e 0,09 g/100 g[11,12].

Inoltre, la porzione di UFA è ottenuta per calcolo dalla differenza tra SFA e acidi grassi totali (stimati 0,95 dei lipidi totali), per cui sono possibili, per singoli campioni, incongruenze tra somma matematica dei singoli valori di MUFA e PUFA e quello totale di UFA. D'altra parte la semplicità della procedura analitica, il ridottissimo costo e la possibilità di analizzare elevati numeri di campioni in rapida sequenza (fino a 400/h), la rende particolarmente idonea a questo tipo di studi in cui la finalità prioritaria è il confronto tra serie di campioni; la stessa metodica è stata del resto già largamente impiegata in lavori similari negli ultimi anni[13,14].

Tabella 1 - Valori medi e deviazione standard dei parametri analitici dei 189 campioni di latte analizzati; confronto con pagamento latte qualità regionale (QMPS).

Parametro (unità di misura)	Media Campioni (Deviazione Standard)	Media QMPS
Grasso (g/100 g)	4,270 (0,602)	3,920
Proteine (g/100 g)	3,470 (0,229)	3,440
Caseina (g/100 ml)	2,720 (0,185)	2,680
Lattosio (g/100 ml)	5,050 (0,152)	5,160
Acidi Grassi Saturi (g/100 g)	2,570 (0,404)	2,389
Acidi Grassi Insaturi (g/100 g)	1,233 (0,272)	1,049
Acidi Grassi Monoinsaturi (g/100 g)	1,129 (0,248)	0,959
Acidi Grassi Polinsaturi (g/100 g)	0,150 (0,040)	0,123
Rapporto Insaturi/Saturi	0,470	0,430
Punto Crioscopico (°C)	-0,527 (0,005)	-0,524
Cloruri EQ (mg/100 ml)	150,060 (18,60)	138,000
Urea (mg/10 ml)	25,310 (7,060)	22,800
Carica Batterica Totale* (UFC/ml)	700.000	45.000
Cellule Somatiche* (cell/ml)	377.000	268.000
Acidità Titolabile (°SH/50 ml)	3,300 (0,310)	N.D.
Indice caseina	0,783 (0,007)	0,777

Note:
*Valore espresso come Media Geometrica
QMPS: Sistema di pagamento del latte in base alla qualità
N.D.: non definita

RISULTATI E DISCUSSIONE

Caratterizzazione generale del latte di montagna

La caratterizzazione complessiva del latte prodotto dagli allevamenti compresi nello studio può essere sintetizzata attraverso i valori medi e le relative deviazioni standard per ciascun parametro (Tabella 1). Nella medesima tabella sono riportati, come generico elemento di confronto, i valori medi osservati nello stesso periodo sui campioni conferiti al laboratorio per il sistema di pagamento del latte in base alla qualità (QMPS) in regione Lombardia, per un totale di circa 4.500 allevamenti in prevalenza di tipo intensivo e dell'area di pianura.

Il quadro risultante fornisce ovviamente soltanto un'immagine generica del latte prodotto dagli allevamenti di montagna nel periodo dello studio; seppur con notevole variabilità (testimoniata dall'elevatissimo valore di deviazione standard di al-

cuni parametri), il latte prodotto dalle diverse mandrie presenta le seguenti caratteristiche principali:

1. livelli di grasso, proteine e caseine decisamente elevati, seppur con ampia variabilità e superiori ai valori medi regionali (considerato il periodo dell'anno e l'effetto della fase di pascolo, ciò è attribuibile alla prevalenza di bovine Bruna Alpina in montagna e la quasi esclusività della Frisona in pianura);
2. contenuto in grasso ed acidi grassi del latte di montagna superiore a quello regionale, così come il rapporto UFA/SFA;
3. notevole differenza di contaminazione batterica che, per quanto riguarda la montagna, è fortemente influenzata dalle condizioni igieniche della produzione e conservazione del latte nel periodo di pascolo.

Composizione degli Acidi Grassi nel latte di montagna tenuto in debito conto dei limiti

Anche per quanto riguarda la composizione delle differenti componenti acidiche, la variabilità osservata risulta consistente: la distribuzione di frequenza per le due componenti principali, UFA e SFA, per i campioni prelevati nel periodo di stabulazione (M in grigio) rispetto a quello di pascolo (AP in nero) è rappresentata nei Grafici 1 e 2.

Anche tramite la dispersione delle medie di allevamento nei due periodi, è apprezzabile graficamente la tendenza alla maggior differenziazione della componente UFA (Grafico 3) rispetto a quella SFA (Grafico 4), nei due periodi di studio.

A conferma delle osservazioni grafiche precedenti, le due serie di dati sono state sottoposte ad un test statistico di significatività delle differenze: test T ($P<0,05$). Il risultato ottenuto è sintetizzato in Tabella 2.

Tenendo in debito conto i limiti di questo tipo di inferenza, riteniamo interessante sottolineare che:

– la componente igienico sanitaria della produzione del latte risulta significativamente peggiorata dal passaggio al pascolo: è facile collegare questi due aspetti alle condizioni di mungitura e conservazione del latte (e dei campioni) per la carica batterica ed all'effetto del cambia-

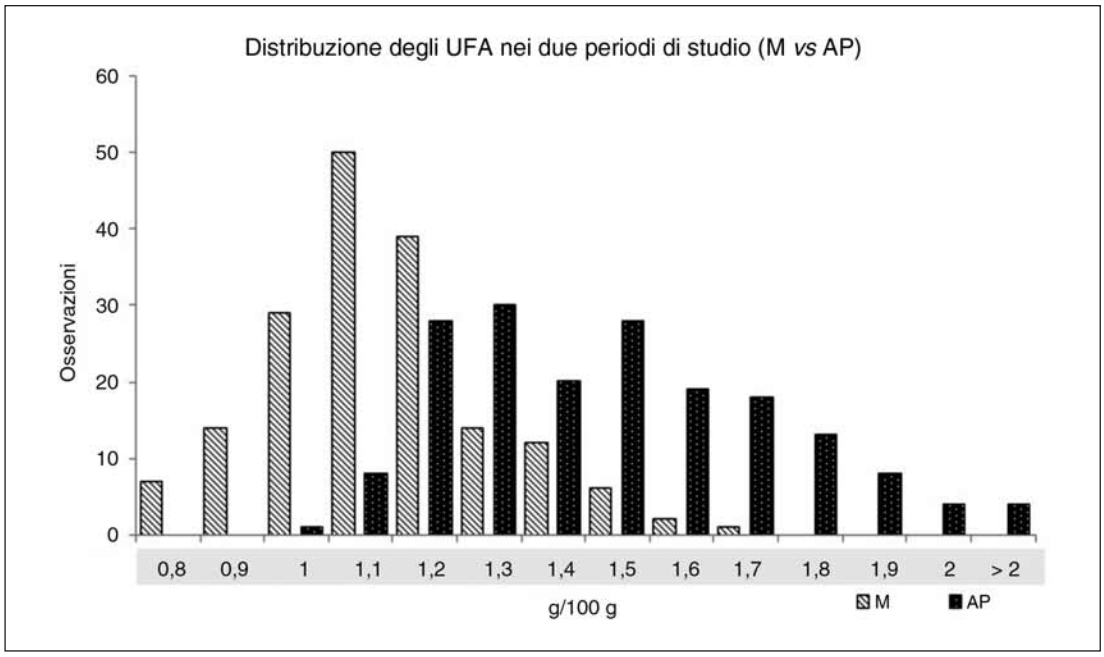

Grafico 1
Distribuzione degli acidi grassi insaturi nei campioni prelevati nel periodo di stabulazione (M) rispetto al periodo di pascolo (AP).

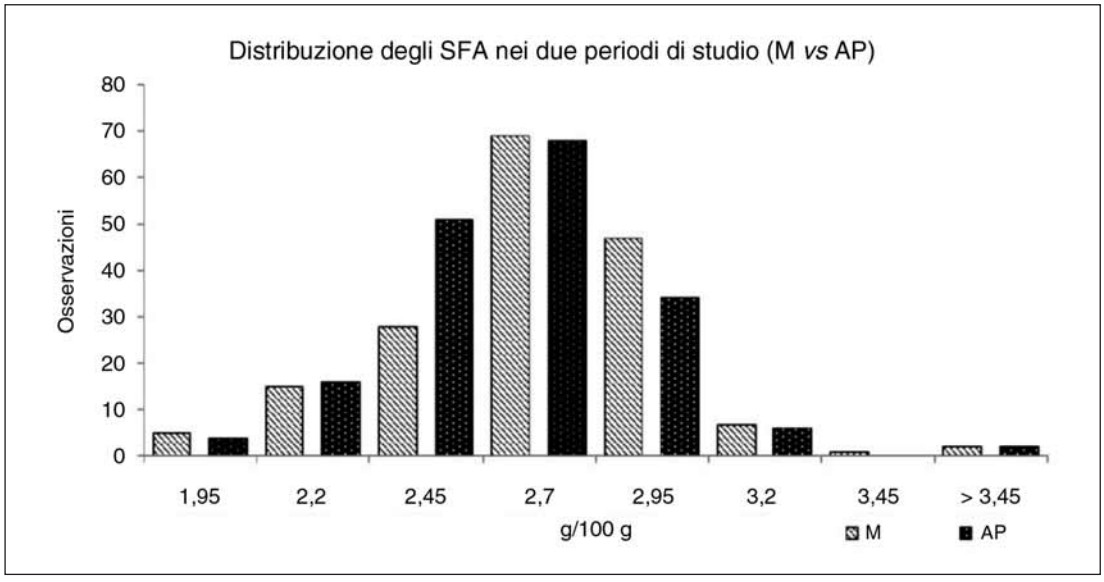

Grafico 2
Distribuzione degli acidi grassi saturi nei campioni prelevati nel periodo di stabulazione (M) rispetto al periodo di pascolo (AP).

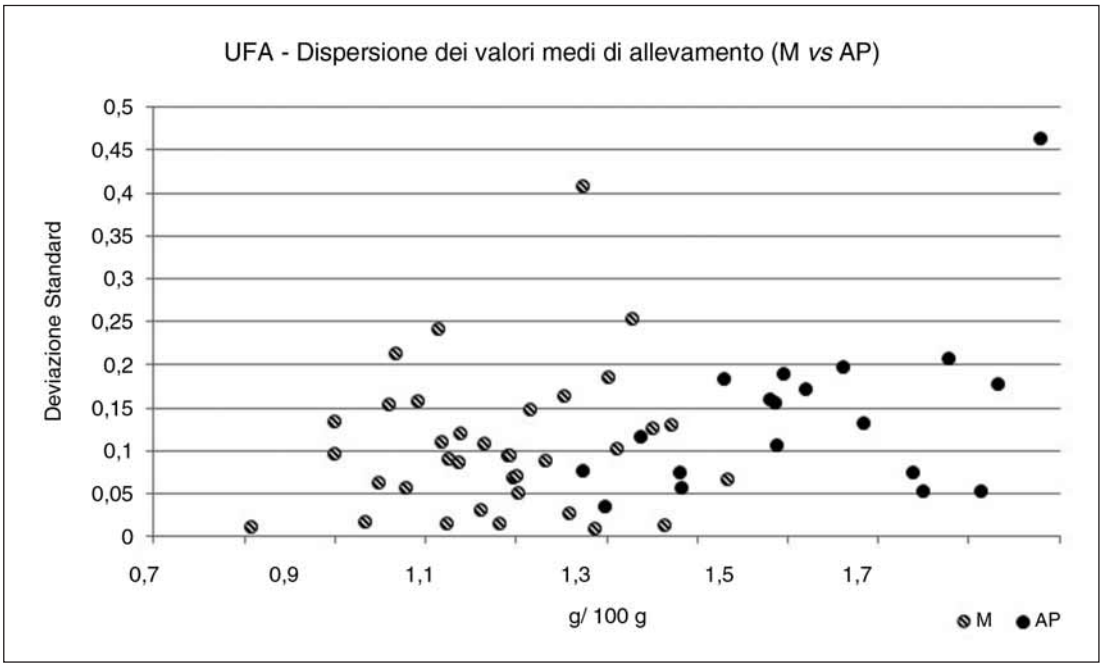

Grafico 3
Dispersione dei valori medi di allevamento in acidi grassi insaturi nel periodo di stabulazione (M) e di pascolo (AP).

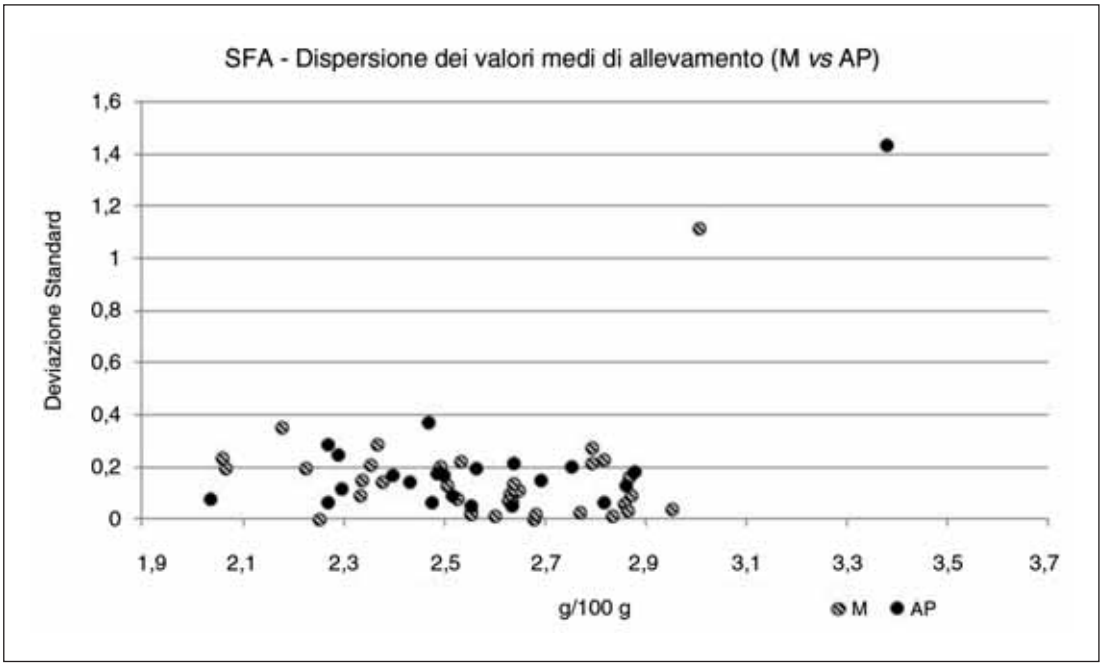

Grafico 4
Dispersione dei valori medi di allevamento in acidi grassi saturi nel periodo di stabulazione (M) e di pascolo (AP).

mento ambientale, climatico e di alimentazione per il tenore in cellule.

– Il valore in grasso risulta incrementato malgrado, in linea generale, l'apporto nutrizionale al pascolo in termini di energia sia inferiore a quello ottenibile in stabulazione. In realtà in alcuni allevamenti dell'area di montagna l'apporto nutritivo nell'ultimo periodo di stabulazione cala fortemente per la limitata disponibilità di scorte di foraggi di buona qualità. Va notato che l'incremento osservato si verifica in un periodo dell'anno in cui negli allevamenti della pianura padana il titolo di grasso e proteine tende tipicamente ad un crollo in relazione al tipico clima caldo umido, evidentemente evitato con il trasferimento al pascolo.
– Risultano interessanti anche le significatività osservate per le componenti di acidi grassi: sebbene il decremento della componente satura risulti statisticamente non significativo, le altre 3 frazioni mostrano tutte incrementi statisticamente significativi; il cambio di regime alimentare sembra dunque incidere non solo sulla quantità, ma anche sulla composizione della frazione lipidica ed in particolare sul rapporto acidi grassi insaturi/saturi.

Considerata la possibilità di interferenze sui dati osservati dovute, come già accennato, al parziale rimescolamento delle mandrie, abbiamo cercato di confermare ulteriormente le precedenti osservazioni selezionando unicamente le 6 malghe in cui gli animali al pascolo risultavano raggruppati in modo corrispondente alle 6 stalle di fondovalle da cui provenivano (Tabella 3).

CONCLUSIONI

Le osservazioni condotte, pur con i limiti citati per quanto riguarda la metodica analitica e la parziale variazione nella

Tabella 2 - Valori medi, deviazione standard e significatività delle differenze tra campioni nel periodo di stabulazione (M) e nel periodo di pascolo (AP).

Parametro (unità di misura)	Media e (DS) M	Media e (DS) AP	Significatività Test T (P< 0,05)
Grasso (g/100 g)	4,133 (0,475)	4,480 (0,643)	Sì
Proteine (g/100 g)	3,471 (0,249)	3,481 (0,201)	No
Cellule Somatiche* (cell/ml)	162.850	340.194	Sì
Carica Batterica* (UFC/ml)	117.700	268.900	Sì
Acidi Grassi Saturi (g/100 g)	2,585 (0,344)	2,541 (0,419)	No
Acidi Grassi Insaturi (g/100 g)	1,088 (0,166)	1,455 (0,259)	Sì
Acidi Grassi Monoinsaturi (g/100 g)	1,004 (0,155)	1,285 (0,246)	Sì
Acidi Grassi Polinsaturi (g/100 g)	0,125 (0,019)	0,176 (0,036)	Sì
Rapporto Insaturi/Saturi	0,420 (0,019)	0,571 (0,044)	Sì

Note:
M: valori inerenti il periodo di stabulazione in valle
AP: valori inerenti il periodo di stabulazione in alpeggio
DS: deviazione standard
*Valore espresso come Media Geometrica

Tabella 3 - Valori medi, deviazione standard e significatività delle differenze tra campioni nel periodo di stabulazione (M) e nel periodo di pascolo (AP), limitatamente alle 6 mandrie rimaste costanti nei due periodi.

Parametro (g/100 g)	Media e (DS) Valori 6M	Media e (DS) Valori 6AP	Significatività Test T (P< 0,05)
Grasso	4,159 (0,366)	4,589 (0,716)	Sì
Acidi Grassi Saturi	2,601 (0,254)	2,600 (0,477)	No
Acidi Grassi Insaturi	1,100 (0,163)	1,500 (0,274)	Sì
Acidi Grassi Monoinsaturi	1,000 (0,150)	1,353 (0,260)	Sì
Acidi Grassi Polinsaturi	0,129 (0,019)	0,182 (0,038)	Sì
Rapporto Insaturi/Saturi	0,420 (0,025)	0,573 (0,048)	Sì

Note:
6M: valori inerenti le 6 mandrie che non hanno subito mescolamenti, nel periodo di stabulazione in valle
6AP: valori inerenti le 6 mandrie che non hanno subito mescolamenti, nel periodo di stabulazione in alpeggio

composizione delle mandrie controllate, offrono un'interessante base informativa per la caratterizzazione del prodotto degli allevamenti nelle aree di montagna ed in particolare per l'apprezzamento dei cambiamenti indotti dal periodo di pascolo in alta quota. Questa nicchia produttiva ha una limitata rilevanza dal punto di vista commerciale rispetto al complesso del settore lattiero-caseario del nord Italia, ma rappresenta un elemento di primaria importanza per l'economia locale, anche per le sue ricadute in termini culturali, sociali e di tutela ambientale. In linea generale riteniamo di aver evidenziato che le specifiche, e spesso difficoltose, condizioni gestionali dei piccoli allevamenti montani consentono di ottenere un prodotto di qualità complessivamente rilevante, adatto alla trasformazione in prodotti caseari tradizionali con alcuni punti di forza ed alcuni punti critici rispetto al prodotto tipico dell'allevamento intensivo di pianura. Per quanto riguarda la composizione in acidi grassi, riteniamo di poter concludere che il regime di pascolo in alta quota costituisce un fattore di significativo miglioramento del rapporto tra le componenti "desiderate ed indesiderate" del latte in relazione alla qualità e salubrità dei prodotti derivati. Questo tipo di osservazioni conferma quanto già evidenziato da altri Autori con studi in molti casi tecnicamente più approfonditi[15,16,17] sui significativi effetti della razione alimentare e, in particolare, di quella caratteristica del pascolo, anche se non limitatamente a quello di alta quota in montagna, sulla composizione in acidi grassi del latte. Anche con tecniche analitiche indirette e decisamente semplici da applicare su elevati numeri di campioni, è possibile contribuire alla valorizzazione ed al sostegno di una filiera produttiva che, oltre alle difficoltà economiche complessive del settore lattiero-caseario dell'ultimo decennio, deve affrontare specifiche sfide di tipo logistico e tecnico.

Pasture influence on fatty acids profile of mountain farms bovine milk

SUMMARY

Introduction - Milk and dairy products are considered an important source of fatty acids in the diet that varies in quantity according to the methods of bovine rearing and feeding. In the last decades, evaluation of the fatty acid composition in bovine milk has been the subject of several studies for the beneficial effects on human health. The aim of this study is to quantify satured and unsatured fatty acids by indirect estimation with Infrared spectroscopy in bovine bulk tank milk samples collected in typical mountain farms, in particular to confirm the effect of pasture period on the milk composition.

Materials and methods - The 189 milk samples were collected from 37 dairy farms representative of the typical "farm" in a mountainous area of North Italy (Valle Camonica): small (12 heads/farm on average), prevalence of Brown Alpine and Red Piebald, low production level (about 16 Lt/head/die), mainly fed with local hay, integration of feedstuffs under 30% of the feed ration, without maize silage. The samples were collected directly from farms (first period, April-May 2015) or from pasture areas (second period, June-July 2015).

Results and discussion - Our observations confirm the change in milk composition related to the grazing period as well with the increased content in fat and unsatured fatty acids and, in particular, the "grazing effect" was statistically confirmed by a significant increase in the unsatured/satured fatty acids ratio: 0.42 (SD 0.025) in farms *vs* 0.57 (SD 0.048) in pasture areas, as already highlighted by other studies made with reference method.

Conclusions - The Fourier transform infrared spectroscopy analytical method, easy to use and cheap, although the indi-

rect evaluation of the milk composition, produces results and information useful to support the quality of milk and milk products in mountain areas, also for larger projects in the future.

KEY WORDS

Milk, fatty acids, FTIR, pasture.

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Bovine papillomatosis in Sicily: virus identification, diagnosis and co-infections

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SUMMARY

Introduction - Bovine papillomatosis is an infectious disease distributed worldwide among cattle herds consisting of hyperproliferative lesions affecting cutaneous tissue and mucosa. The disease, known as papillomas, may occur in different part of the body. Bovine papillomaviruses (BPVs) are non-enveloped double stranded DNA viruses belonging to the *Papillomaviridae* family, a group of viruses that infect all amniotes. Despite the benign characters of the lesions, in some cases bovine papillomatosis may dramatically reduce the productive performances when the lesions are extensive over the body involving teats and udders.

Aim - This work was particularly aimed at the identification and characterization of BPVs circulating in Sicily in order to obtain autovaccine against BPVs.

Material and methods - Thirty-one epithelial lesions, collected from cattle, clinically diagnosed as papillomas, were referred to the Laboriatories of Istituto Zooprofilattico Sperimentale of Sicily.

Samples were analyzed by electron microscopy, PCR amplification, Rolling Circle Amplification, sequencing, negative staining electronic microscopy and standard histological examinations.

Results and discussion - The investigation revealed the presence of different BPV types 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, mostly in the form of co-infections. Besides BPV amplification, zoonotic viruses belonging to the genus parapoxvirus within the *Poxviridae* family were screened and detected in 11 samples. This is the first study performed in Sicily, reporting a wide diversity of BPV genera and types circulating in the territory.

Conclusion - The data collected showed the necessity of proper diagnosis to produce an effective vaccine while virus characterization is important to know the epidemiological scenario of the region and set appropriate control measures and reliable diagnostic methods for bovine papillomatosis. The presence of co-infecting zoonotic viral agents underlies the need for a better understanding of the possible interactions between the two viruses and suggests the importance of identifying proper measures to prevent the infection to humans.

KEY WORDS

Papillomavirus; diagnosis; autovaccine; Parapoxvirus; co-infections.

INTRODUCTION

Bovine papillomaviruses (BPVs) are non-enveloped ds DNA viruses causing hyper proliferative benign tumors localized on skin and mucosa of cattle.

According to Bernard and colleagues (2010), papillomaviruses responsible for bovine infections are classified in four genera: *Deltapapillomavirus* (BPV-1/2/13), *Epsilonpapillomavirus* (BPV-5/8), *Xipapillomavirus* (BPV-3/4/6/9/10/11/12) and *DyoXipapillomavirus* (BPV-7) but the development of sensitive detection methods and more intensive research on animal papillomaviruses have allowed the identification of novel viral types. Further eight putative BPVs have recently been identified: BPV-14 classified as *Deltapapillomavirus,* BPV 15, 17, 20 classified as *Xipapillomavirus*, BPV 16, 18 classified as *Dyokappapapillomavirus* and BPV 19 and 21 which have yet to be assigned to genera and subgroups (Papillomavirus Episteme (PaVE); http://pave.niaid.nih.gov). BPVs infect keratinocytes inducing epithelial papillomas while *Deltapapillomaviruses* determine fibropapillomas of paragenital areas, skin, alimentary tract and urinary bladder. Cutaneous lesions normally regress in healthy animals, but they can also result in economic losses in both beef and dairy production. Papillomas in teats and udders hamper cow milking, and may promote secondary infections leading to mastitis.

At present no BPV treatment is available and application of surgical excision of papillomas is not affordable in large herds with a high prevalence. Despite a number of studies on vaccines, there are no commercial formulations against BPVs. The presence of multiple BPV types and variants in the same

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lesion have been already reported[2,3,4] and suggests the need of multivalent VLP (virus like particles) vaccines. Otherwise herd specific vaccine seems to be the easiest practice to confer protection against the disease caused by different BPV types even though its efficacy needs to be experimentally proved.
In this light the knowledge of the BPV epidemiological situation in an area is critically relevant to set up proper control measures. The aim of this study was to investigate the aetiologic agents causing bovine papillomatosis in clinical specimens collected from different provinces in Sicily. Together with BPVs, the presence of zoonotic parapoxviruses was investigated as a previous study[5] reported the co-infection of *Papillomaviridae* and *Poxviridae* in cutaneous lesions of domestic ruminants in Sicily. The parapoxvirus genus comprises two viral species infecting cattle, Bovine papular stomatitis virus (BPSV) and Pseudocowpox virus (PCPV). The viruses infect via damaged skin and give rise to proliferative and/or ulcerative lesions of the skin and mucosae and can infect humans.

MATERIALS AND METHODS

A total of thirty-one clinically diagnosed papillomas were collected from teats, udders, head and neck by field veterinary practitioners in different provinces of Sicily. Samples were submitted to Istituto Zooprofilattico Sperimentale Laboratories (IZS) of Sicily for the production of BPV Herd-Specific Vaccines.
Negative staining Electronic Microscopy (EM) was performed on each sample.
Standard histological examinations were conducted on 18 formalin fixed samples that were processed and stained by hematoxylin and eosin method (HE). Slides were evaluated microscopically and histopathologic findings were classified as fibroapilloma or papilloma.
Viral DNA was purified from frozen pathological samples (NucleoSpin® Tissue, MN) according to manufacturer's instructions. The quality of the extracted DNA was checked through the amplification of the bovine β-globin gene. Diagnostic PCR and RCA followed by sequencing and enzymatic digestion were performed to detect and identify the BPV genera and types in each sample. A subset of BPVs type and genus specific primer pairs were used as already described by Savini and collaborators[6]. Amplification was also performed by multiply-primed Rolling-Circle Amplification (RCA) using the Illustra TempliPhi 100 amplification kit (GE Healthcare, Little Chalfont, UK) following the protocol previously described by Rector and collaborators[7]. A digestion with restriction enzymes BamHI and HindIII was subsequently performed to confirm the presence of DNA fragments consistent with the length of a papillomaviral genome. All specimens were also subjected to amplification by Pan-parapoxvirus primers[8] to detect parapoxviruses (PPV) known to be endemic in the area. Purified PCR amplicons were sequenced using the Sanger method (Perkin-Elmer Applied Biosystems) and chromatograms were assembled (4 Peaks 1.7.1).

RESULTS

Negative-staining EM showed the presence of particles resembling BPVs in size and shape in thirty samples out of thirty-one while not any parapoxvirus particle was identified with this technique. PCR showed a single infection with BPV in only three lesions while the remaining 28 harbored two (6 lesions) or three (22 lesions) different BPV types. Sequence analysis and RCA results demonstrated the presence of BPV 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12. In particular, twenty-nine samples harbored BPV1 or 2 (*Deltapapillomavirus* genus) confirming to be the most widespread BPV types in cattle. A 97% identity with the nucleotide sequence of BPV-4 (GenBank accession n. X05817.1) was shown in one sample through sequencing of the 400 base pair amplified fragment of the L1 gene. BPV-5 and BPV-8 belonging to the genus *Epsilonpapillomavirus* were detected in three different samples while *Xipapillomavirus* was the second genus mostly detected in the cutaneous samples (20/31) and BPV-6 was the most represented (11/20). *DyoXipapillomavirus* (BPV-7) was detected in seven out of thirty-one specimens. In addition, RCA amplification and digestion lead to the identification of patterns consistent in size with BPVs in 27/31 samples. Restriction enzyme profiles of digested RCA product allowed the identification of BPV type in 8 samples while in the other 19 samples the results were not always in accordance with PCR findings, as co-infections were detected only in few cases, but most of the time pattern was consistent with the presence of only one papillomaviral genome.
Coinfection with zoonotic parapoxvirus was detected in 11 samples, sequencing of the PCR products identified 7 PCPV and 4 BPSV. Histologically, lesions were classified as fibropapillomas (14 samples) and papillomas (4 samples). Various degrees of hyperplasia of the epidermis with irregular papillary projections into the dermis were observed in all samples. Papillomas showed a core of dermal stroma that supported the proliferating epithelium. Capillaries within the dermis were dilated and congested. The *stratum corneum* exhibited variable degrees of orthokeratotic to parakeratotic hyperkeratosis. The cells of upper spinous layer showed swollen lightly basophilic cytoplasm (ballooning degeneration), eccentric pyknotic nuclei and a perinuclear halo, referred to as koilocytes, the hallmark of BPV infection. The stratum granulosum had large, variably sized and shaped basophilic keratohyaline granules, often larger than normal. Microscopic lesions typical of fibropapillomas showed acanthosis, hyperkeratosis and down growth of rete ridges, but dermal proliferation was the main feature (Figure 1). The proliferating cells were large, plump fibroblasts, arranged in haphazard whorls and fascicles. The epidermal proliferation was minimal and only as slight acanthosis and accentuation of rete pegs was seen.
Despite PCR amplification of zoonotic poxviruses in 11 samples, histologic examination revealed typical signs of parapoxvirus infection only in one PCPV and one BPSV-positive fibropapilloma samples: keratinocyte ballooning degeneration was prominent and scattered, large, eosinophilic cytoplasmic inclusions were observed (Figure 2).

DISCUSSION

In this study the identification of the aetiological agents of clinically diagnosed papillomavirus lesions collected in different cattle farms of Sicily was performed. The presence of the virus is usually assumed by clinical examination only,

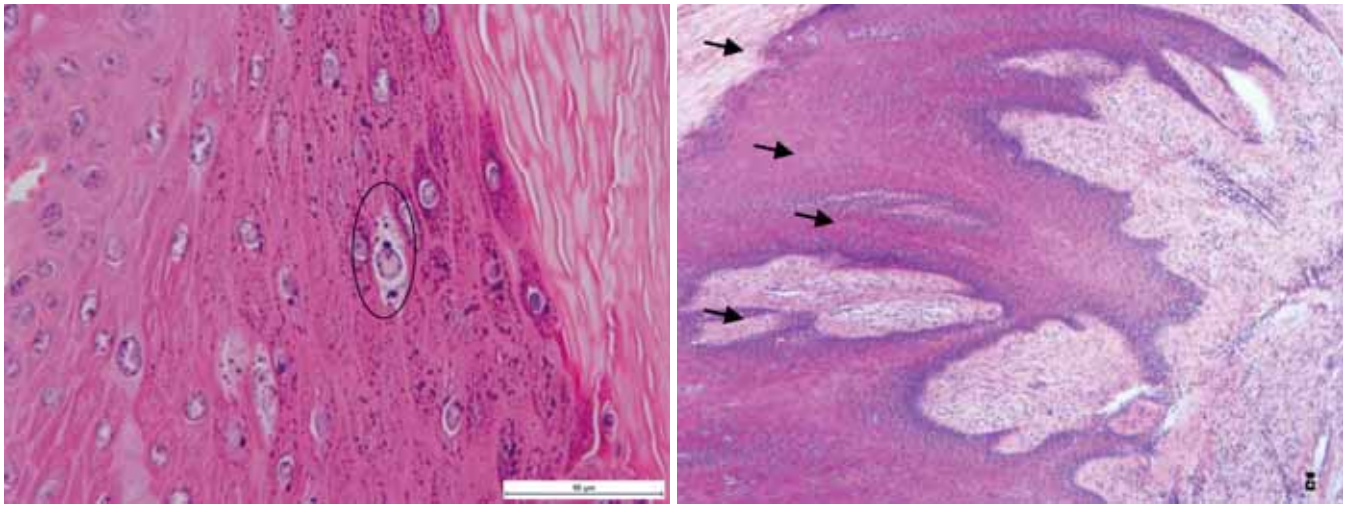

Figure 1 - Koilocytes (ellipse), basophilic keratohyaline granules in a lesion classified as papilloma (left). Wartlike filiform exophytic proliferation of hyperkeratotic stratified squamous epithelium supported by mature fibrovascular stalks in a fibropapilloma (right). H&E. Bar= 50 µm.

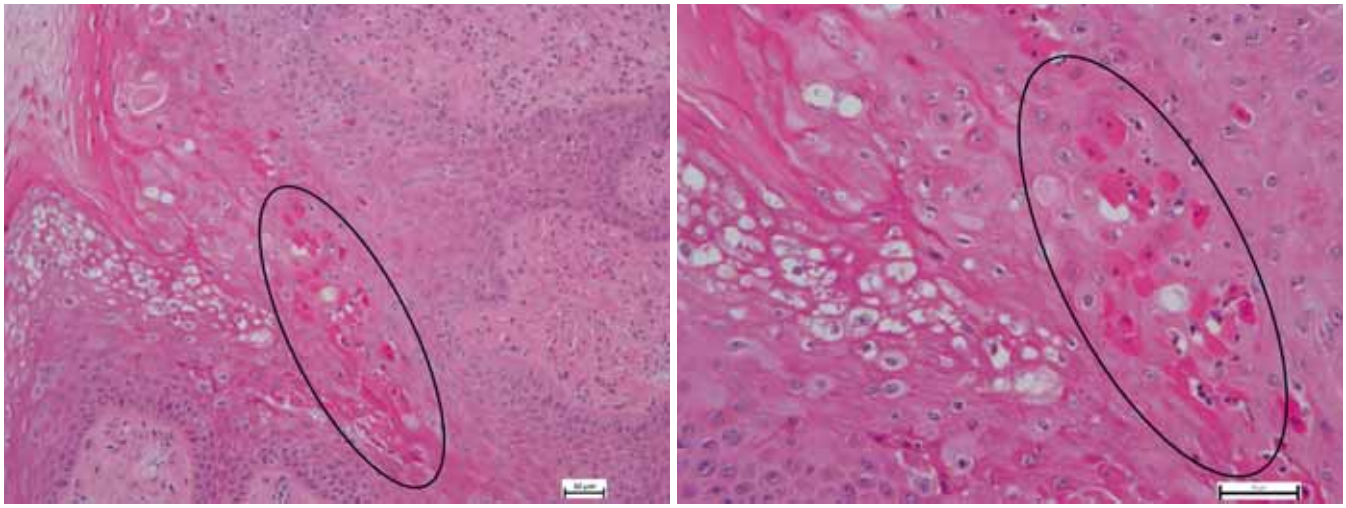

Figure 2 - Ballooning degeneration of keratinocytes of stratum spinosum containing basophilic intracytoplasmic inclusion bodies (ellipse) typical of parapoxvirus infection. H&E. Bar= 50 µm.

while studies investigating BPVs' diversity are sporadic therefore scarce information about distribution of types in Italy is available[9,6].

In all cases the specimens collected were referred as "papilloma" because the animals were showing multiple proliferative lesions disseminated throughout the whole body and may involve a single animal or most of the herd. To date, different papillomavirus types and genera have been identified as causative agents of papillomas, in particular 21 different BPV types belonging to five different papillomavirus genera have been identified. The combination of different amplification strategies and sequencing permitted to detect most of the BPV types and genera officially classified by ICTV. Nucleotide sequencing confirmed the presence of BPV-4 in one papilloma. This infection was reported by Borzachiello and collaborators[10] and was associated to Oesophageal papillomas in cattle that feed on bracken fern present in the south of Italy. BPV-4 and BPV-5 weren't detected in other two recent studies aimed at identification and characterization of BPV types circulating in North west Italy[9] and in Emilia-Romagna region[6] but we don't know if it is imputable to different diagnostic methods used for the investigations or to the real different widespread of the two BPV types on the Italian territory. Except for two samples, at least two but more frequently three different type of BPVs were detected in the lesions confirming that co-infection of different BPVs in the same specimen is a frequent finding in field samples. It remains a subject of speculation whether the warts are caused only by one or more BPVs type, but it is remarkable the need of a proper diagnostic tool able to detect all different BPVs especially when no information are available on the viral types circulating in a certain area. At present mixed BPVs infection have been discovered using generic or genus specific primers that may fail in the detection of novel BPVs. RCA is a sensitive method for the detection of novel BPVs and the use of specific primers is not requested, but after amplification digestion by restriction enzyme, cloning and sequencing are frequently needed for the BPV type identification, becoming a labor-intensive and time consuming technique for routine diagnosis. On the other hand, the identification of specific viral types present in the papillomatous lesions is fundamental for appropriate vaccines development like herd-specific vaccination. In particular, therapeutic vaccine are requested from the farmers in Sicily because despite the

proposal of several vaccine models[11,12] as no commercial products are available to prevent BPV infections. As observed by Batista and colleagues[13], no connections were demonstrated among involved viral types and various clinical and histopathological findings particularly in case of coinfections. *Deltapapillomavirus* have always been related to cutaneous fibropapilloma and *Xipapillomaviruses* to epithelial papilloma[14] moreover Grindatto and collaborators[9] classified BPV1 infected samples as squamous papilloma and specimens coinfected by BPV1 and other BPV types as fibropapilloma. The finding of BPV1 or BPV2 in 93.5% of the specimens confirmed the histological diagnosis of fibropapilloma. Moreover, despite the low sensitivity of ME, negative staining was able to detect papillomavirus particles in all samples except one suggesting feasibility of therapeutic vaccine preparation even though its efficacy in inducing early regression of warts is still questionable[15]. The observation of eosinophilic inclusion bodies representing the viral factories of parapoxviruses, suggests the possibility that these zoonotic agents coinfecting papillomatous lesions are biologically active. Up to now the detection of viruses belonging to the Poxviridae family was obtained only by molecular biology. Even though the virus viability still needs to be proved these data poses major questions about different viruses' interaction, prevention of zoonotic transmission and supports the recommendation for clinical diagnoses by laboratory analyses before preparation of herd specific vaccine.

CONCLUSION

This is the first study aiming at detection and characterization of BPVs circulating in Sicily. The work confirms what was previously observed about the high frequency of co-infections in papillomatous lesions of cattle and demonstrates the wide diffusion of different BPVs on the Italian territory remarking the need of a standard rapid diagnostic tool for identification of BPV types in cattle infections. Moreover, clinical diagnosis needs to be confirmed by laboratory analysis in light of the further reporting of the presence of zoonotic viruses coinfecting papillomatous lesions in cattle and representing a potential source of infection for humans when animals are handled.

CONFLICT OF INTEREST STATEMENT

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RIASSUNTO DELLE CARATTERISTICHE DEL PRODOTTO Denominazione del medicinale veterinario: Imrestor 15 mg soluzione iniettabile per bovini. **Composizione:** Ogni siringa preriempita da 2,7 ml contiene: principio attivo, Pegbovigrastim (fattore bovino di stimolazione delle colonie di granulociti pegilato, PEG bG-CSF) 15 mg **Forma farmaceutica:** Soluzione iniettabile. Soluzione limpida, da incolore a giallo pallido. **Specie di destinazione:** Bovini (vacche da latte e manze). **Indicazioni:** Come coadiuvante in un programma di gestione dell'allevamento, volto a ridurre il rischio di mastite clinica nelle vacche da latte e nelle manze nel periodo del periparto, durante i 30 giorni successivi al parto. **Controindicazioni:** Non usare in caso di ipersensibilità al principio attivo o ad uno degli eccipienti. **Avvertenze speciali:** In una sperimentazione europea sul campo, l'incidenza di mastite clinica osservata nel gruppo trattato era del 9,1% (113/1235) e nel gruppo di controllo del 12,4% (152/1230), mostrando una relativa diminuzione dell'incidenza della mastite del 26,0% (p=0,0094). L'efficacia è stata testata nel corso della normale pratica di gestione dell'allevamento. La mastite clinica viene identificata attraverso modificazioni dell'aspetto del latte o del quarto mammario o del latte e del quarto insieme. **Precauzioni speciali per l'impiego.** *Per gli animali:* Solo per somministrazione sottocutanea. In uno studio sulla sicurezza in vacche di razza Jersey, il margine di sicurezza di questo prodotto è stato di 1,5 volte la dose massima raccomandata (era stato somministrato un sovradosaggio di 60 µg/kg in tre occasioni). Non eccedere la dose raccomandata. *Per la persona che somministra il medicinale veterinario:* In caso di autoiniezione accidentale, possono insorgere cefalea e dolore osseo e muscolare. Potrebbero anche insorgere altri effetti, inclusi nausea, eruzione cutanea e reazioni di ipersensibilità (difficoltà di respirazione, ipotensione, orticaria e angioedema). Rivolgersi immediatamente ad un medico mostrandogli il foglietto illustrativo o l'etichetta. **Posologia e via di somministrazione:** *Somministrazione sottocutanea.* Il regime di trattamento è composto da due siringhe. Il contenuto di una singola siringa preriempita deve essere iniettato per via sottocutanea in una manza/vacca da latte 7 giorni prima della data prevista del parto. Il contenuto di una seconda siringa preriempita deve essere iniettato per via sottocutanea entro le 24 ore successive al parto. L'intervallo tra le due somministrazioni non deve essere inferiore a 3 giorni o superiore a 17 giorni. Una singola siringa fornisce una dose di 20-40 µg/kg di pegbovigrastim a seconda del peso corporeo. **Sovradosaggio:** Evidenze derivanti dall'uso di principi attivi simili nell'uomo suggeriscono che la somministrazione accidentale di una dose maggiore a quella autorizzata potrebbe causare reazioni avverse, correlate all'attività di pegbovigrastim. Il trattamento deve essere sintomatico. Non esiste alcun antidoto noto. **Reazioni avverse (frequenza e gravità):** Negli studi clinici, sono state osservate reazioni anafilattoidi non tipiche con frequenza non comune. Le vacche presentavano gonfiore delle membrane mucose (principalmente a livello di vulva e palpebra), reazioni cutanee, aumento della frequenza respiratoria e della salivazione. In casi rari, l'animale può collassare. Questi segni clinici di solito compaiono da 30 minuti a 2 ore dopo la prima dose e si risolvono nel giro di 2 ore. Può rendersi necessario un trattamento sintomatico. La somministrazione sottocutanea del prodotto potrebbe provocare gonfiore locale transitorio nel sito di iniezione, nonché reazioni infiammatorie che si risolvono entro 14 giorni dal trattamento. **Impiego durante la gravidanza e l'allattamento:** può essere usato durante la gravidanza e l'allattamento. **Interazione con altri medicinali veterinari e altre forme d'interazione:** La somministrazione contemporanea di sostanze che alterano la funzione immunitaria (ad es.corticosteroidi o farmaci antinfiammatori non steroidei) potrebbe ridurre l'efficacia del prodotto. L'uso contemporaneo di tali prodotti deve essere evitato. **Tempi di attesa:** carne e visceri: zero giorni. latte: zero giorni. **Speciali precauzioni per la conservazione:** Conservare in frigorifero (2 °C – 8 °C). Non congelare. Sensibile alla luce. Conservare nella confezione originale. Il prodotto può essere conservato a 25 °C per un massimo di 24 ore. **Titolare dell'autorizzazione all'immissione in commercio:** Eli Lilly and Company Limited, Elanco Animal Health. Priestley Road, Basingstoke, Hampshire RG24 9NL. Regno Unito. **Numero dell'autorizzazione all'immissione in commercio:** EU/2/15/193/001-003. **Regime di dispensazione:** Da vendersi soltanto dietro presentazione di ricetta medico-veterinaria in copia unica non ripetibile.

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Interbreed variation in craniometrical parameters in sheep

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SUMMARY

This work is the first investigation to compare craniometrical parameters between different breeds of sheep. Nine breeds or cross-breeds of sheep were studied, with all measurements being carried out on live animals at times of routine animal husbandry. Although a number of the variables were not significantly different between breeds, examples of interbreed differences were found. This was true for both individual measurements of lengths and widths, as well as for indices derived by pair-wise comparisons of individual widths or lengths. In some cases differences indicated that the cross-bred lambs shared a greater similarity to one of the parental breeds relative to the other parental breed; e.g. the width between the ears relative to the length from the nuchal crest to the rostral aspect of the upper lip, where Texel x Wiltshire cross lambs had ratios more similar to the maternal Wiltshire than they had to the paternal Texel line. In other examples the cross-breeds had values which were intermediate between the two parental lines; e.g. the width at the rostral aspect of the diastema relative to the length from the nuchal crest to the rostral aspect of the upper lip, where Texel x Wiltshire cross lambs had ratios intermediate between the maternal Wiltshire and the paternal Texel lines. For one of the ratio values (the length from the nuchal crest to the rostral aspect of the upper lip, relative to the width at the caudal aspect of the diastema) it was particularly interesting to note that this was a trait where the male Suffolk x Brecknock Hill Cheviot cross lambs were more similar to the Suffolks (i.e. the paternal line), but the females cross lambs were more similar to the Brecknock Hill Cheviots (i.e. the maternal line). It is anticipated that this work will provide implications for using craniometrical parameters as a future husbandry tool.

KEY WORDS

Sheep; craniometrical parameters; interbreed comparisons.

INTRODUCTION

Recording data from domesticated animals is a key component of modern husbandry and welfare for both agricultural animals and pets. Examples of measurements routinely taken vary between species but include variables such as: birthweight; weaning weight; withers height, etc.

Craniometry is a long-established discipline in anthropology as evidenced by a book review by Oetteking from over 100 years ago[1]. Although originating in human biology it has been applied to other species. Particularly in dogs, measurements have been made regarding variables in skull shape (e.g.[2]), with skull measurements being used for purposes such as historical breed changes, behavioural traits and general health. For example, studies have shown historical changes to skull shapes of St Bernards[3] as well as links between head measurements and dog trainability[4]. Based on cephalic indices dog breeds can be classified into 3 general groups; brachycephalic (e.g. pugs with relatively flat faces), dolichocephalic (e.g. greyhounds with relatively long faces) or mesaticephalic (e.g. collies with intermediate length faces). However, recently other skull diversity factors have also been studied[5].

There has been interest in skull measurements in ruminants, e.g. Spanish ibex[6], goats[7-9], roe deer[10] and sheep. Interest in sheep heads involved behavioural analysis[11] as well as anatomical abnormalities. There has also been some work carried out on head measurements as part of larger whole-body investigations[12], limited investigations of comparisons between two sheep breeds[13-14], and work on multiple measurement points for skulls[15-17], on single breeds in each paper. Geographic features of the British Isles have led to numerous breeds of domesticated animals, often based on thriving better on different grounds (e.g. fast-growing animals on lowlands, versus hardier animals on uplands). This has led to over 60 native breeds of sheep within the British Isles[18], together with several breeds imported from mainland Europe and a range of cross-breeds by crossing purebred lines. Initially geographical isolation and establishing flock books led to discrimination between breeds based on morphological characteristics, although more recently they have been distinguished by genetic markers (e.g.[19]). Although breed criteria include morphological characteristics such as body size, there has generally been little consideration of skull shape, as it probably has little impact on major selection criteria e.g. meat production or wool quality.

There are few papers including sheep craniometrical measurements, and with two exceptions using two Turkish sheep breeds[13-14], information on interbreed craniometrical measurements has not underpinned publications. This work investigates head measurements to analyse differences in head morphology in different sheep breeds, and compares measurements in cross lambs relative to parental lines.

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MATERIALS AND METHODS

Animals used and measurements made

Three different groups of animals had craniometrical measurements taken. In each case all animals within a group had measurements determined by a single author at a time of routine husbandry work; prior to livestock being sold at market. Measurements (see Table 1) were made using measuring tapes rather than callipers, as they allowed rapid but accurate measurements with minimal stress to animals which were not used to being handled.

Group 1 - Craniometrical measurements were made using 3 groups of adult ewes (≥ 18 months); 30 Southdown, 30 Ryeland and 30 Suftex (Suffolk x Texel crosses). All measurements, 3 lengths (L1 to L3) and 2 widths (W1 and W2) were recorded by a single author (CEB).

Group 2 - Craniometrical measurements were made using 3 groups of adult ewes (≥ 18 months); 20 Wiltshire Horn (WH), 20 Texel and 20 WH x Texel crosses. All measurements, 3 lengths (L1 to L3) and 4 widths (W1 to W4) were recorded by a single author (RD).

Group 3 - Craniometrical measurements were made using 3 groups of lambs aged around 6 months; 50 Brecknock Hill Cheviot (BHC), 50 Suffolk and 50 BHC x Suffolk crosses - 25 males and 25 females per group. All measurements, 5 lengths (L1 to L5) and 4 widths (W1 to W4) were recorded by a single author (MHO).

Statistical Analysis

Within groups data were checked for normal distribution before interbreed comparisons using F-test supported student T-tests. In addition, a series of indices were derived using one measurement divided by another, and expressed as the first variable as a percentage of the second. This was performed for all length: width and width: width combinations, and the statistical analysis repeated as above.

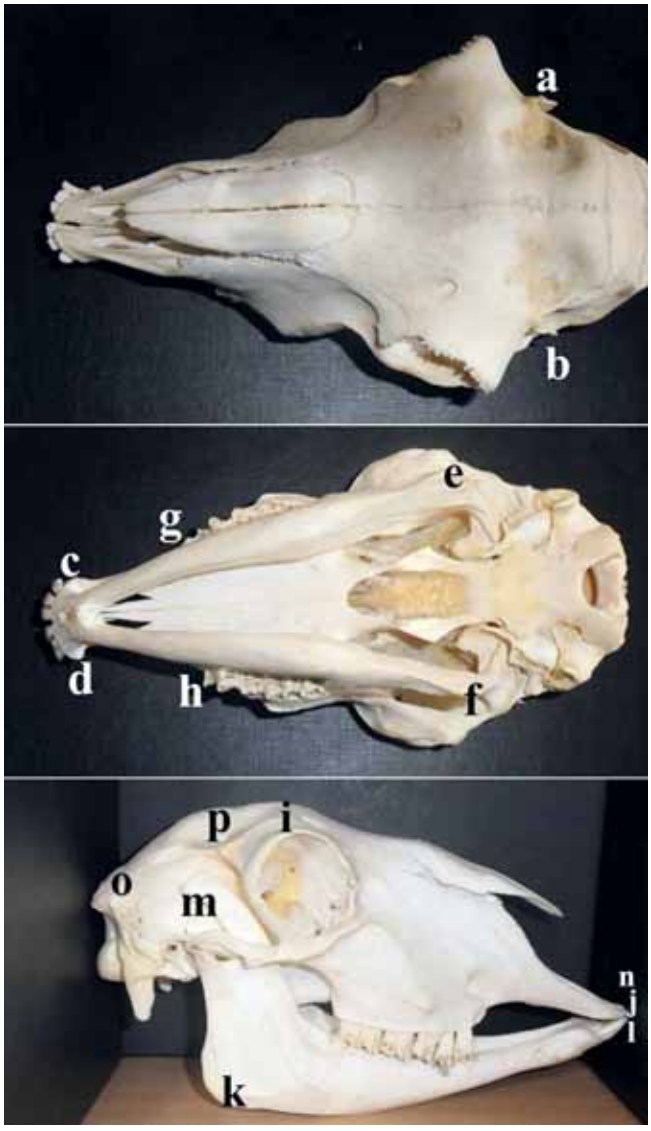

Figure 1 - Positions at which measurements were made on the sheep heads. Note that all measurements were made in live animals but this image uses a skull to make viewing easier.

RESULTS

Group 1

Data for adult Southdown, Ryeland and Suftex ewes are shown in Table 2. This shows that sizes of some features did not vary significantly between breeds. For example, the length from the nuchal crest to the rostral aspect of the upper lip [L1] was not significantly different between breeds. However, other measurements did vary, e.g. width between the ears [W1] was significantly different between breeds. Furthermore, the length from the caudoventral aspect of the

Table 1 - Variables measured within the current work. The symbols denote the position shown on the image in Figure 1.

		Symbol 1	Symbol 2
W1	Width between the ears	a	b
W2	Width at rostral aspect of diastema - between outer incisors (I4) of left and right jaws	c	d
W3	Width between caudal aspects of left and right jaws	e	f
W4	Width at caudal aspect of diastema - between first premolars (P1) of left and right jaws	g	h
L1	Length from nuchal crest to rostral aspect of upper lip	i	j
L2	Length from caudoventral aspect of angle of mandible to rostral aspect of lower lip	k	l
L3	Length from caudal aspect of hinge of lower jaw to nasal tip	m	n
L4	Length from akrokranion to rostral aspect of upper lip	o	j
L5	Poll to the rostral aspect of upper lip	p	j

Table 2 - Five measurements (in mm) recorded for the Ryeland, Southdown and Suftex (Suffolk x Texel) adult ewes (n=30 for each breed), together with the corresponding indices which have been derived by expressing the size of the first variable as a percentage of the second variable. SEM values are shown in parenthesis. Values within rows which are not significantly different (P>0.05) share a superscript.

	Ryeland	Southdown	Suftex
W1	154 [a] (2.4)	143 [b] (1.6)	126 [c] (2.2)
W2	76 [a] (1.5)	84 [b] (2.5)	121 [c] (2.3)
L1	244 [a] (3.5)	239 [a] (5.8)	239 [a] (2.6)
L2	140 [a] (3.9)	149 [ab] (4.1)	158 [b] (2.3)
L3	175 [a] (3.8)	173 [a] (3.4)	208 [b] (2.3)
W1 - L1	63 [a] (1.4)	61 [a] (1.4)	53 [b] (0.9)
W1 - L2	114 [a] (3.4)	99 [b] (2.5)	80 [c] (1.8)
W1 - L3	90 [a] (2.4)	84 [a] (1.8)	61 [b] (1.1)
W2 - L1	31 [a] (0.7)	35 [b] (0.7)	51 [c] (0.9)
W2 - L2	56 [a] (1.9)	57 [a] (1.3)	77 [b] (1.8)
W2 - L3	44 [a] (1.3)	49 [a] (1.1)	58 [b] (1.2)
W1 - W2	206 [a] (4.5)	176 [b] (4.6)	105 [c] (2.3)

Table 3 - Seven measurements (in mm) recorded for the Texel, Wiltshire Horn and Texel x Wiltshire Horn adult ewes (n=30 for each breed), together with the corresponding indices which have been derived by expressing the size of the first variable as a percentage of the second variable. SEM values are shown in parenthesis. Values within rows which are not significantly different (P>0.05) share a superscript.

	Texel	Wiltshire	Texel x Wiltshire
W1	146 [a] (1.4)	151 [a] (2.8)	144 [a] (1.8)
W2	74 [a] (1.6)	73 [a] (1.2)	73 [a] (1.1)
W3	107 [a] (2.3)	107 [a] (2.2)	104 [a] (2.2)
W4	94 [a] (1.7)	94 [a] (1.7)	99 [a] (1.3)
L1	230 [a] (2.0)	259 [a] (1.4)	247 [a] (1.8)
L2	185 [a] (2.5)	172 [a] (2.4)	172 [a] (2.6)
L3	178 [a] (2.9)	178 [a] (4.7)	166 [a] (1.8)
W1 - L1	63 [a] (0.6)	59 [b] (1.1)	58 [b] (0.7)
W1 - L2	79 [a] (0.9)	88 [b] (1.7)	84 [ab] (1.6)
W1 - L3	83 [a] (1.3)	87 [a] (2.3)	87 [a] (1.5)
W2 - L1	32 [a] (0.6)	28 [b] (0.5)	30 [ab] (0.5)
W2 - L2	40 [a] (0.8)	43 [a] (0.9)	43 [a] (1.0)
W2 - L3	42 [a] (1.4)	42 [a] (1.3)	44 [a] (0.7)
W3 - L1	51 [a] (1.0)	41 [b] (0.8)	42 [b] (1.0)
W3 - L2	63 [a] (1.2)	62 [a] (1.5)	61 [a] (1.7)
W3 - L3	66 [a] (1.5)	61 [a] (1.7)	63 [a] (1.1)
W4 - L1	46 [a] (0.8)	37 [b] (0.7)	40 [c] (0.6)
W4 - L2	57 [a] (1.2)	55 [a] (1.2)	58 [a] (1.4)
W4 - L3	60 [a] (1.6)	55 [a] (1.8)	59 [a] (0.8)
W1 - W2	201 [a] (4.2)	213 [a] (6.8)	199 [a] (4.3)
W1 - W3	126 [a] (2.2)	145 [b] (3.9)	140 [b] (3.7)
W1 - W4	140 [a] (2.9)	165 [b] (5.2)	148 [ab] (3.2)
W2 - W3	63 [a] (1.2)	70 [ab] (1.8)	71 [b] (1.2)
W2 - W4	70 [a] (1.1)	78 [b] (1.0)	74 [ab] (0.8)
W3 - W4	112 [a] (2.0)	115 [a] (3.0)	106 [a] (1.8)

mandible angle to the rostral aspect of the lower lip [L2] was significantly longer in Suftexs ($\bar{x}$=158 mm, SEM=2.3) than Ryelands ($\bar{x}$=140 mm, SEM=3.9), but neither differed significantly from that of the intermediate Southdowns ($\bar{x}$=149 mm, SEM=4.1).

Group 2

The data for adult Texel, WH and Texel x WH cross ewes are shown in Table 3. Differences in absolute values for these breeds were not significant (P>0.05).

However indices derived from these measurements showed significant differences. In some indices the parental breeds were significantly different, but cross ewes had intermediate values and were not significantly different from either parent. One such an index was the ratio between L1 (length from nuchal crest to aspect of upper lip) relative to W1 (width between ears).

In other indices, cross ewes were not significantly different from WHs, but both cross ewes and the WHs were significantly different from Texels e.g. L1 (length from nuchal crest to aspect of upper lip) relative to W3 (width between caudoventral aspects jaws).

There were no indices where crosses showed similarity to Texels but not to the WHs.

One index, W4 (width between caudal aspect of diastema) relative to L1 (length from nuchal crest to rostral aspect of upper lip) was significantly different in all 3 breeds, with values for crossbreds intermediate between those for pure breeds.

Finally, there was an example where Texels and WHs were not significantly different, but cross ewes were significantly different from either pure breed. This was seen when comparing W2 (width at rostral aspect of diastema) relative to W3 (width between caudoventral aspects of jaws). It was assumed that this observation was due to proportions of the Texel and WH head shapes being similar, but that proportions in crosses were different.

Group 3

All animals were around 6 months old when measured. At this age some measurements were already different, although this could be due to developmental differences between breeds, rather than interbreed differences in mature animals.

For example the width between the ears [W1] differed based on a combination of breed and gender factors, with male Suffolks having the largest means ($\bar{x}$=154 mm, SEM=2.8) and female BHCs having the smallest mean value ($\bar{x}$=90 mm, SEM=1.3).

Examples were seen where cross lambs had values more similar to those of the BHCs than those of Suffolks. For example the length from the caudal aspect of the hinge of the lower jaw to the nasal tip [L3], where differences were not significant between crosses and BHCs (P>0.05), irrespective of gender (mean lengths ranged from 178-181 mm). However this value was significantly longer in Suffolk females ($\bar{x}$=187 mm, SEM=1.6), and males ($\bar{x}$=206 mm, SEM=3.2).

The opposite was seen in other examples. The index derived by comparing L2 (length from caudoventral aspect of angle of mandible to rostral aspect of lower lip) relative to W2 (width at rostral aspect of diastema) was not significantly different (P>0.05) in Suffolk lambs and cross lambs with males ($\bar{x}$=26 in both breeds) and females ($\bar{x}$=25 in both

breeds). However the mean BHC values were significantly shorter (23 and 21 for males and females respectively).

As well as interbreed differences, gender differences were observed. For example the length from the nuchal crest to the rostral aspect of the upper lip [L1]. In this case male BHCs ($\bar{x}$=224 mm, SEM=2.1) and male crosses ($\bar{x}$=218 mm, SEM=2.5) were not significantly different (P>0.05), and female BHCs ($\bar{x}$=208 mm, SEM=2.8) and female crosses ($\bar{x}$=208 mm, SEM=2.3) were not significantly different (P>0.05). However both lengths in males were significantly (P<0.05) longer than in females.

Indices were also observed with differences between breeds and genders. For example the ratio between W4 (width at caudal aspect of diastema) and L1 (length from nuchal crest to rostral aspect of upper lip). Male and female Suffolks, ($\bar{x}$=22, SEM=0.7 and $\bar{x}$=22, SEM=0.7 respectively) did not differ significantly (P<0.05). Likewise male and female BHCs, ($\bar{x}$=20, SEM=0.4 and $\bar{x}$=20, SEM=0.5 respectively)

Table 4 - Nine measurements (in mm) recorded for the Suffolk, Brecknock Hill Cheviot and Suffolk x Brecknock Hill Cheviot lambs (25 males and 25 females for each breed), together with the corresponding indices which have been derived by expressing the size of the first variable as a percentage of the second variable. SEM values are shown in parenthesis. Values within rows which are not significantly different (P>0.05) share a superscript.

	Suffolk		Brecknock Hill Cheviot		Suffolk x Cheviot	
	Male	Female	Male	Female	Male	Female
W1	154 ^{a} (2.8)	131 ^{b} (2.1)	102 ^{c} (1.8)	90 ^{d} (1.3)	138 ^{e} (2.2)	112 ^{f} (2.6)
W2	44 ^{a} (1.12)	39 ^{b} (1.0)	36 ^{c} (0.7)	32 ^{d} (0.6)	41 ^{b} (0.7)	38 ^{c} (0.5)
W3	97 ^{a} (2.0)	87 ^{b} (1.7)	78 ^{c} (1.3)	74 ^{d} (1.2)	84 ^{b} (1.6)	69 ^{e} (1.2)
W4	58 ^{a} (1.2)	55 ^{b} (1.4)	44 ^{c} (0.9)	41 ^{d} (0.8)	49 ^{e} (1.1)	42 ^{cd} (0.7)
L1	266 ^{a} (5.8)	249 ^{b} (3.8)	224 ^{c} (2.1)	208 ^{d} (2.8)	218 ^{c} (2.5)	208 ^{d} (2.3)
L2	176 ^{a} (3.6)	160 ^{bc} (2.4)	160 ^{bc} (1.8)	156 ^{bde} (1.7)	160 ^{cd} (2.1)	151 ^{e} (1.8)
L3	206 ^{a} (3.2)	187 ^{b} (1.6)	178 ^{c} (1.9)	178 ^{c} (1.5)	181 ^{c} (2.4)	178 ^{c} (1.3)
L4	346 ^{a} (4.8)	323 ^{b} (3.2)	273 ^{c} (3.4)	259 ^{d} (3.0)	271 ^{c} (4.1)	273 ^{c} (2.6)
L5	228 ^{a} (2.82)	213 ^{b} (2.3)	191 ^{c} (1.6)	180 ^{d} (1.3)	190 ^{c} (1.6)	187 ^{c} (1.6)
W1 - L1	58^{a} (1.5)	53 ^{b} (1.2)	46 ^{c} (0.9)	44 ^{c} (0.9)	63 ^{d} (1.3)	54 ^{b} (1.4)
W1 - L2	88 ^{a} (2.9)	83 ^{a} (1.9)	64 ^{b} (1.3)	58 ^{c} (1.0)	86 ^{a} (1.7)	74 ^{d} (1.9)
W1 - L3	75 ^{a} (1.7)	70 ^{b} (1.4)	57 ^{c} (1.1)	51 ^{d} (0.9)	77 ^{a} (1.6)	63 ^{e} (1.4)
W1 - L4	45 ^{a} (0.8)	41 ^{b} (0.7)	37 ^{c} (0.6)	35 ^{d} (0.7)	51 ^{e} (1.)	41 ^{b} (1.0)
W1 - L5	68 ^{a} (1.1)	62 ^{b} (1.2)	54 ^{c} (1.2)	50 ^{d} (0.8)	73 ^{e} (1.3)	60 ^{b} (1.4)
W2 - L1	17 ^{ab} (0.6)	16 ^{a} (0.5)	16 ^{a} (0.4)	16 ^{a} (0.4)	19 ^{c} (0.4)	18 ^{bc} (0.3)
W2 - L2	26 ^{a} (1.0)	25 ^{a} (0.6)	23 ^{b} (0.5)	21 ^{c} (0.4)	26 ^{a} (0.5)	25 ^{a} (0.4)
W2 - L3	22 ^{ab} (0.6)	21 ^{a} (0.6)	20 ^{a} (0.4)	18 ^{c} (0.3)	23 ^{b} (0.4)	21 ^{a} (0.3)
W2 - L4	13 ^{a} (0.3)	12 ^{a} (0.4)	13 ^{a} (0.3)	13 ^{ab} (0.3)	15 ^{c} (0.3)	14 ^{b} (0.2)
W2 - L5	19 ^{ab} (0.5)	19 ^{ac} (0.6)	19 ^{ac} (0.4)	18 ^{c} (0.3)	22 ^{d} (0.4)	20 ^{b} (0.3)
W3 - L1	37 ^{ab} (1.2)	35 ^{ac} (0.9)	35 ^{ad} (0.7)	36 ^{a} (0.7)	39 ^{b} (0.8)	33 ^{cd} (0.7)
W3 - L2	55 ^{a} (1.2)	55 ^{a} (1.1)	49 ^{b} (1.0)	48 ^{bc} (0.8)	53 ^{a} (1.1)	46 ^{c} (1.0)
W3 - L3	47 ^{a} (1.0)	47 ^{a} (1.0)	44 ^{b} (0.7)	42 ^{c} (0.7)	47 ^{ab} (1.1)	39 ^{d} (0.7)
W3 - L4	28 ^{a} (0.7)	27 ^{a} (0.6)	29 ^{a} (0.7)	29 ^{a} (0.5)	31 ^{b} (0.7)	25 ^{c} (0.5)
W3 - L5	43 ^{ab} (0.9)	41 ^{a} (1.1)	41 ^{a} (0.7)	41 ^{a} (0.7)	44 ^{b} (0.9)	37 ^{c} (0.6)
W4 - L1	22 ^{a} (0.7)	22 ^{a} (0.7)	20 ^{b} (0.4)	20 ^{b} (0.5)	22 ^{a} (0.5)	20 ^{a} (0.5)
W4 - L2	33 ^{a} (0.9)	35 ^{a} (1.0)	28 ^{bc} (0.6)	26 ^{b} (0.5)	30 ^{d} (0.7)	28 ^{c} (0.5)
W4 - L3	29 ^{ab} (0.7)	30 ^{a} (0.9)	25 ^{c} (0.5)	23 ^{d} (0.4)	27 ^{b} (0.8)	24 ^{cd} (0.5)
W4 - L4	17 ^{abc} (0.5)	17 ^{ac} (0.5)	16 ^{abd} (0.2)	16 ^{be} (0.4)	18 ^{c} (0.5)	16 ^{de} (0.3)
W4 - L5	26 ^{a} (0.6)	26 ^{a} (0.8)	23 ^{b} (0.5)	23 ^{b} (0.4)	26 ^{a} (0.7)	23 (0.4) ^{b}
W1 - W2	266 ^{a} (6.9)	241 ^{b} (5.4)	232 ^{bc} (4.3)	223 ^{c} (5.8)	286 ^{a} (7.6)	266 ^{a} (7.3)
W1 - W3	352 ^{a} (8.6)	337 ^{a} (8.5)	284 ^{b} (5.4)	281 ^{b} (5.3)	337 ^{a} (6.4)	299 ^{b} (8.7)
W1 - W4	161 ^{ab} (4.7)	152 ^{a} (3.5)	131 ^{c} (3.3)	122 ^{d} (2.3)	165 ^{b} (3.8)	162 ^{ab} (4.3)
W2 - W3	47 ^{ab} (1.2)	46 ^{a} (0.8)	47 ^{ab} (0.9)	44 ^{a} (0.6)	50 ^{b} (0.9)	54 ^{c} (0.8)
W2 - W4	77 ^{ab} (1.4)	71 ^{a} (1.3)	81 ^{cd} (1.3)	80 ^{bc} (1.2)	87 ^{de} (1.3)	89 ^{e} (1.3)
W3 - W4	167 ^{ab} (3.7)	160 ^{a} (4.4)	179 ^{c} (4.7)	182 ^{c} (3.9)	174 ^{bc} (3.7)	165 ^{ab} (3.4)

were not significantly (P<0.05) different. However male crosses ($\bar{x}$=22, SEM=0.5) were more similar to Suffolks and female crosses ($\bar{x}$=20, SEM=0.5) were more similar to BHCs. A similar pattern was seen for the index derived from W4 (width at caudal aspect of diastema) relative to L5 (length from poll to top lip); male crosses being more similar to Suffolks and female crosses being more similar to BHCs.

DISCUSSION

Previous studies of sheep craniometrical measurements tend to have concentrated on using skulls without soft tissue. The two exceptions to this[13-14], were part of a wider range of measurements across the body. Therefore, as far as we are aware, this is the first comparison of craniometrical measurements between breeds in a single paper, and the first analysis of multiple head measurements in live sheep. The current work uses data collected by three individuals from different sites. Therefore no comparison has been made between groups, to avoid potential inter-experimenter variation. In addition, groups 1 and 2 involved adult animals, but group 3 used younger animals, again meaning comparisons across all groups were not appropriate. Repeating measurements by a single experimenter could have allowed comparisons between groups, but animals were measured during routine handling after which some animals were sold, meaning they were not available for retrospective measurements.

The work involved measurements with a tape, rather than callipers as these sheep were not used to being handled, and so minimised stress levels with rapid recording of data. However SEM values within groups (Tables 2 to 4) were all relatively low, suggesting low variability within single samples, supporting the assumption that tapes gave adequately reliability.

The first set of analysis were performed primarily to demonstrate that differences (e.g. W1) between breeds could be detected using a measuring tape with low variability. Therefore measurements undertaken were the five which were felt could be made rapidly and would involve minimal stress to the animals. Using these values it was possible to demonstrate that statistically significant (P<0.05) interbreed differences could be detected in terms of absolute values, and also in indices derived from these measurements. Moreover, statistical analysis of these identified statistically significant differences. In this example, two of the breeds were unrelated purebred lines, and the third breed was a cross which was not derived from either of these. Thus no relationship comparisons were performed between the three sets of animals.

The second analysis used two pure breeds; Texels and WHs, and a cross derived by mating Texel rams and WH ewes. A further two measurements were included, again ones which it was felt could be made rapidly and with minimal stress. As above only adult ewes were used. This group was used to examine if particular distances or indices in the crosses were more similar to those seen in specific parents. Two indices were not significantly (P>0.05) different in the WHs and crosses, but significantly different (P<0.05) in Texels relative to the other breeds; ratio of the width between the ears [W1] relative to width between the caudoventral aspects of jaws [W3], and ratio of the width at the rostral aspect of the diastema [W2] relative to width at the caudal aspect of the diastema [W4]. The values in the cross ewes were more similar to those in WH ewes, suggesting these traits may show a genetic bias.

In this group there were also two examples where crosses had intermediate values, which were not significantly different (P>0.05) from either parental breed, but both parental breeds differed significantly (P<0.05) from each other; ratio of the length from the nuchal crest to the rostral aspect of the upper lip [L1] relative to width at the rostral aspect of the diastema [W2], and ratio of width between the ears [W1] relative to width at the caudal aspect of the diastema [W4].

Both of the first two groups used data from adult ewes, meaning potential gender differences were not considered. Primarily this was due to individual farms generally have enough adult ewes available to allow comparisons, but insufficient adult rams to permit this. In mammals males tend to be larger and heavier, a factor which prompted the third study involving males and female from two pure breeds (BHCs and Suffolks), and crosses produced from BHC ewes and Suffolk rams. However, routinely insufficient males are retained for breeding, meaning analysis were restricted to animals at 6 months, rather than adults as above. A further two measurements were included at this point, again ones which could be carried out rapidly and with minimal stress.

The length from the nuchal crest to the rostral aspect of the upper lip [L1] in male BHC lambs ($\bar{x}$=224 mm, SEM=2.1) and male crosses ($\bar{x}$=218 mm, SEM=2.5) were not significantly different, and those in female BHC lambs ($\bar{x}$=208 mm, SEM=2.8) and female cross lambs ($\bar{x}$=208 mm, SEM=2.3) were also not significantly different, suggesting this length in the cross lambs had more in common with the equivalent length in BHC lambs of the same sex.

The length from the caudal aspect of the hinge of the lower jaw to the nasal tip was not significantly different between the BHC and cross lambs; irrespective of gender. Neither of these factors was observed in Group 2 study using Texels, WHs and their cross.

It is worth noting that the mean length from the akrokranion to the rostral aspect [L4] of the upper lip and the mean length from the poll to the top lip [L5], measurements not recorded in the previous two groups, both showed significant similarity in length in cross lambs of both sexes relative to the equivalent distance in male BHC lambs.

Some indices provided interesting comparisons for preliminary comparisons between groups - albeit they were carried out by different individuals. For example the ratio of width between the ears [W1] relative to width between the caudoventral aspects of left and right jaws [W3], where Texel x WH crosses were more similar to WHs (maternal line) than Texels, but BHC x Suffolk crosses (both genders) showed more similarity to male Suffolks (i.e. the paternal lineage).

The ratio between the length from the nuchal crest to the rostral aspect of the upper lip [L1] and width between the ears [W1] was not significantly different (P>0.05) between Suffolk females and female BHC x Suffolk crosses. This pattern was repeated for the length from the poll to the top lip [L5] relative to width between the ears [W1], with similar values between Suffolk females and female BHC x Suffolk crosses, again similarity with the maternal lineage.

In other cases there was similarity between Suffolk males and male BHC x Suffolk crosses. There was no significant difference in ratios between the length from the caudal as-

pect of the hinge of the lower jaw to the nasal tip [L3] relative to width between the ears [W1], and the length from the caudoventral aspect of the angle of the mandible to the rostral aspect of the lower lip [L2] relative to width between the ears [W1].

The ratio of width at the rostral aspect of the diastema to the length from the caudoventral aspect of the angle of the mandible to the rostral aspect of the lower lip was similar in both the Suffolk lambs and BHC x Suffolk crosses. However the values for the BHCs showed significant ($P<0.05$) gender differences. This group identified potential gender differences for one index; ratio of the length from the nuchal crest to the rostral aspect of the upper lip [L1] relative to width at the caudal aspect of the diastema [W4], where male crosses were more similar to Suffolks (both genders), but the female crosses more similar to BHCs (both genders).

The full implications of this work are unclear as this is the first investigation in sheep on this scale. The role of craniometrical parameters in understanding problems in dogs has been clear for some time (e.g.[2]) with more detailed recent studies (e.g.[5]), particularly the role of head shape to health issues such as birthing and respiratory problems in brachycephalic dogs. However factors affecting heads are being implicated as important in understanding pain and stress in sheep[20].

It is in these areas that analysing craniometrical parameters, and estimating what constitutes normal, may have a future impact on sheep breeding. In some sheep breeds head size and shape can pose lambing problems. It has been estimated that dystocia accounts for around half of deaths in lambs in the first 72 hours after birth[21], i.e. around 7% of lambs born. Although lambing difficulties vary between breed and are not restricted to the lamb's head, these pose major problems at lambing time, particularly when numbers are considered at both national and international levels.

Furthermore, some sheep breeds, as with some brachycephalic dogs, are prone to respiratory and breathing problems e.g. laryngeal chondritis. Therefore an exploration of a potential linkage between head morphology and traits such as respiratory problems later in life is worth further exploration.

In conclusion, this work is the first to carry out interbreed comparisons of a range of craniometrical parameters in live sheep. Examples of differences were found between breeds, including some suggesting that some parameters in the cross breeds are more similar to one of the parental breeds than the other, and also examples of intermediate mean values. Moreover, differences within breeds could be seen between male and female lambs, including an example where the male cross lambs showed more similarity to the paternal breed and the ewe lambs showed more similarity to the maternal breed.

Nine breeds, or crosses of breeds were studied in the current work, and up to nine measurements were made for each breed. These data for variation between breeds in terms of head measurements and craniometrical indices derived from them, may argue for head measurements becoming a topic worth investigating in more detail, using more measurements than those used in the current work. Moreover the range of domesticated breeds which exist internationally, coupled with other species in the *Ovis* genus and other genera in the Caprinae Subfamily, argue that there is scope for further craniometric measurements in other animals to understand these relationships.

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Monitoring of selected milk quality parameters during lactation of a Mediterranean dairy sheep breed

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SUMMARY

Introduction - Sheep and goats are the most efficient transformers of low quality forage into high quality animal products (milk and meat) with distinguished characteristics. Sheep and goat milk production in several Mediterranean countries is mainly taking place in less favoured, rural and mountainous areas. In Greece, sheep production is of high importance for mountainous, arid, semi-mountainous and semi-arid areas, in which sheep are exploited under extensive or semi-extensive management regimes. In those areas, the use of autochthonous breeds is preferred. Arta sheep breed (known as Frizarta) is a local breed reared in the North-Western areas of Greece. Milk composition is a key factor that influences milk quality, and in turn the ratio processed dairy products and farm economics.

Material and methods - The aim of the present study was to monitor selected milk quality parameters during lactation of the Arta sheep breed in a less favoured, rural and mountainous area of North-Western Greece. A total of 840 randomly selected ewes from the Arta breed (8 different flocks), managed under semi-extensive production system, were examined during several lactation stages (months) over three consecutive years. Milk was analysed for somatic cell count and fat, protein, lactose, solids-non-fat content.

Results and discussion - Lactation stage significantly altered milk composition but not somatic cell count. Lactose was negatively correlated with somatic cell count as well as fat and protein content and positively with solids-non-fat content. There was a negative correlation between somatic cell count and solids-non-fat content. The average annual milk yield for the first, second and third year was 250, 260 and 280 kg respectively with no statistical significant differences among years.

Conclusions - The results indicate that milk quality of the Arta breed is good. The good milk quality can support the production of local traditional cheeses. Locally made dairy products of added value can in turn positively affect the income of farmers and contribute to the endurance of rural human population in less favoured areas.

KEY WORDS

Arta breed, dairy ewes, less developed, Mediterranean, milk composition.

INTRODUCTION

At the European level, the dairy sheep population is confined, at a large part, to the Mediterranean countries. The intensification and dairy specialization processes have taken place throughout the whole Mediterranean area especially in countries such as France and Spain and to a lesser extent in Greece[1]. Sheep and goat milk production in several Mediterranean countries is mainly situated in less favoured, rural and mountainous areas[1]. More specifically, Greece, and Italy and a few other countries, are characterised as countries with a high proportion of mountains considering the high percentage of utilised agricultural area designated as less favoured areas[2].

Ewe's milk is widely used in the Mediterranean and Balkan regions for the production of cheese, yoghurt and other dairy products. Furthermore, milk composition is of great importance since it affects the quality and determines the ratio "processed product/milk" and therefore the cost. Improving milk yield and quality of sheep milk in less developed, rural and mountainous areas is of paramount importance in order to increase farming economics. The composition of sheep milk varies widely in relation to breed, nutritional and environmental factors, stage of lactation, parity and season[3,4,5,6,7,8,9]. More specifically, in the semi-extensive production systems of dairy sheep in Greece, the feeding is based mainly on grazing native pastures whose composition depends on climatic conditions and seasonality. In such feeding regimes, the animal nutritional requirements may be under or over met which affect the milk yield and chemical composition[10]. Moreover, in these farming productions systems the milking is, in most cases, operated manually which also

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has an impact on milk quality. Thus, the aim of the present study was to monitor the milk quality of the Arta sheep breed which is farmed in a less favoured, rural and mountainous area of North-Western Greece.

MATERIALS AND METHODS

Characteristics of Arta breed

The Arta sheep breed, also known as Frisarta, was developed in Greece more than 40 years ago based on a breeding program supported and monitored by the Ministry of Rural Development and Food aiming to the genetic improvement of the indigenous sheep population. It was developed with the objective of improving milking potential and prolificacy of indigenous sheep. Following an intensive breeding selection program that involved crossing East Friesian rams with an indigenous sheep population.

The greatest concentration of these sheep is on the plains of Arta in Western Greece. Arta ewes are of large size and body weight (63.3±7.8 kg) with white hair and a long thin tail[11,12]. It is a dairy breed with average annual milk yield of about 230-250 kg/head, making it one of the highest milk producing breeds in Greece[13]. Milk yield is peaked in April and reduced by late July to a level which is no longer commercially viable. The average herd size ranges between 50 to150 ewes and is considered relatively small. Husbandry and herd management (feeding regiments, health status and confinements) are evaluated as satisfactory.

Management of the sheep

The study was conducted on the plains of Arta prefecture, located in North-Western Greece. A total of 840 dairy sheep of Arta breed, from 8 different flocks of the same typical feeding and management practices, were used. Lambing started in mid-November and lasted about 2 weeks. The lambs were then suckling their mothers approximately 45 days. The milking of ewes started at the beginning of January.

Sheep nutrition was based mainly on supplementary feeding during the winter months, up to mid-March while from April onwards grazing native pastures was the only source of feed. The native pastures consisted predominantly of graminaceae and leguminosae and to a smaller extend of papilionacea and compositae. The supplementary feeding consisted of concentrates plus alfalfa hay and straw. The composition of the concentrate diet (g/kg) which was given to sheep was the following: maize, 450; barley, 154; wheat middlings, 235; soybean meal, 130; $CaCo_3$, 24; NaCl, 5 and vitamin and mineral premix, 2.

Milk records and samples were obtained fortnightly for three consecutive lactation periods. Samples of milk were analysed for fat, protein, lactose, solids-non-fat (SNF) using i.r. spectrophotometer (Milko Scan, model 6000; Foss Electric, Hillerod, Denmark). Furthermore, somatic cell count (SCC) in milk was performed by a Fossomatic 6000 (Foss Electric) apparatus using the standard of the International Dairy Federation[14].

Statistical analysis

Statistical analysis was performed using statistical package SPSS (version 14.0, Chicago, IL, USA) and results are presented as mean ± root mean square error. Somatic cell counts were log10 transformed. Kolmogorov-Smirnov test revealed that all data followed normal distribution. Differences in chemical composition and count of somatic cells of ewe's milk between lactation stages over the three consecutive years were tested using repeated measures analysis of variance (ANOVA) with two factors, lactation stage and year. Multiple comparisons were obtained using Tukey's test. Pearson's correlation coefficients were used to determine relationships between the examined physicochemical properties. For all tests a P-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Milk characteristics

Mean values of milk composition and SCC at different lactation stages (months) are shown in Table 1. Lactation had a significant effect on all main constituents ($P<0.05$), except lactose and LogSCC. In detail, the fat content of milk exhibited a gradual decrease from the beginning of the lactation period, reaching a minimum in the middle of lactation (spring) followed by a constant increase until the end of lactation in July. A similar variation pattern for fat composition

Table 1 - Composition and count of somatic cells of ewe's milk per lactation over a period of three consecutive years.

Factor studied	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	LogSCC
Lactation (month)					
January	6.75^{ab}	5.84^{b}	4.85	11.37^{b}	5.92
February	6.90^{b}	5.81^{b}	4.92	11.39^{b}	5.94
March	6.19^{a}	5.68^{ab}	5.03	11.35^{b}	5.89
April	6.20^{a}	5.46^{ab}	5.02	11.19^{ab}	5.90
May	6.18^{a}	5.53^{ab}	4.94	11.16^{ab}	5.95
June	6.79^{ab}	5.39^{a}	4.88	11.04^{a}	6.07
July	7.12^{b}	5.56^{ab}	4.83	11.10^{ab}	6.00
Year					
1st	6.65	5.51^{a}	4.92	11.14^{a}	6.07
2nd	6.59	5.66^{b}	4.93	11.21^{ab}	5.92
3rd	6.59	5.73^{b}	4.93	11.33^{b}	5.86
RMSE	0.33	0.35	0.14	0.34	0.16
Source of Variation	P value				
Lactation	0.036	0.008	NS	0.001	NS
Year	NS	0.002	NS	0.008	NS
Lactation x Year	0.037	NS	NS	0.032	0.029

Data are means of eight replicates (n=8). Means within a column, within a main effect comparison, with different superscripts are different at $P<0.05$. NS: non significant; SNF: solids-non-fat; SCC: Somatic cell count; RMSE: root mean square error.

has been reported by Voutsinas et al.[15]. Average fat content was lower compared to the one reported by Boyazoglu and Morand-Fehr[7] for the Greek breeds of Chios, Vlahiko, Karagouniko and Attiki, but higher than that reported for milk of some breeds in Mediterranean countries[16,17,18]. Similar results have been reported for the Arta breed and Chios breed, respectively[11,19]. Lower fat content in spring was due to lower NDF, ADF content of the diet (grass).

Protein content of milk progressively decreased to a minimum in June followed by a gradual increase until the end of the lactation. Season can affect milk fat and protein and in hot, humid months fat and protein content can be depressed possibly attributed to changes of feed intake patterns which may be lower in summer due to changes in weather and temperature. The above results are in agreement with the results reported by Papavasiliou [11] for the same breed, similar to the ones reported for the Greek breed of Chios[19] and for Massese ewes[16], but lower compared to those reported for other Greek breeds[15,20]. Overall, average protein content was higher than that reported in foreign breeds[16,21].

Lactose content of milk, did not exhibit statistically significant alterations as lactation progressed although was 3% higher in spring compared to summer values. A similar pattern has been reported in similar studies[22,23]. Similar results were reported for the Chios breed by Ploumi et al.[19]. Average lactose content was similar to those reported for various Greek[11,15,20] and foreign breeds[16].

The SNF content progressively decreased to a minimum in June compared to the beginning of lactation. The results were similar to those reported for the Chios breed[18] but lower than that reported for other Greek breeds[15,20].

The LogSCC varied, however not significantly, throughout the lactation. In general, the levels of the SCC in the milk of the Arta breed were similar to those reported in other dairy sheep breeds[16,18]. Previous studies have shown that the stage of lactation and hand milking have an impact on SCC[7,24,25]. Hand-milking, like that implemented in the present study, has been reported to provide an efficient and uniform stripping and reduce variability[17].

In addition to the milk constituents reported above, average annual milk yield for the first, second and third year was 250, 260 and 280 kg respectively with no statistical significant differences among years. Furthermore, some physicochemical characteristics were determined including milk density, pH and acidity (data not shown). The average milk density was almost identical to that reported for several Greek breeds[15,20]. Average pH was higher compared to that reported in the literature[16,26], but similar to that reported for the Greek breed of Boutsiko[15]. Average acidity was similar to that reported by others[20,26].

It should be noted that the reasons for the conflicting findings of various researchers regarding the pattern of variation of milk constituents are not clear, but it is possible that they may be due to differences in the feeding regiments of the ewes[27], the milking procedures, the breeding or in the climate[28].

Correlations between milk constituents

Pearson correlation coefficients among the different variables investigated are presented in Table 2. Most of the variables were correlated. Lactose was negatively correlated with fat, protein and LogSCC and positively correlated with SNF. These findings are in accordance with the findings of Simos et al.[20] and Sinapis[24]. There was a negative correlation between LogSCC and SNF which agrees with the findings reported by others[29,30,31]. Fat was positively correlated with protein and negatively correlated with lactose.

Table 2 - Correlations between the examined physicochemical properties of Arta's sheep milk.

	Fat	Protein	Lactose	SNF
Protein	0.33***			
Lactose	-0.45***	-0.22**		
SNF	0.16*	0.82***	0.19*	
LogSCC	0.27***	-0.01	-0.32***	-0.16*

Level of statistical significance of each of the pairwise correlations, *P≤0.05; **P≤0.01; *** P≤0.001

The present study revealed that overall the milk composition of the Arta dairy sheep breed is good and within the range reported in the literature. Since milk composition is a key factor for milk quality and in turn affects quality of produced dairy products, preservation or further improvement of milk quality may support farmers' income in less favoured, rural areas.

Under this context, several other factors may affect farmer's income and activity in less favoured rural areas that need to be taken into account. More specifically, under recent changes in the Common Agricultural Policy (CAP), low-quality land areas which have traditionally been used as grazing lands are not eligible anymore for European Union income support payments, thus farmers' income may be negatively affected[32]. In order to increase endurance of rural human population in less favoured areas, undertake of other economic activities should take place. The most promising one is on-farm cheese manufacturing of locally made dairy products. Production of local traditional dairy products, like cheeses, is an expression of people's culture and lifestyle, and a reflection of the history of the local area because traditional cheese manufacture is a result of accumulated empirical knowledge passed from generation to generation[33,34]. The scheme of production of local traditional cheeses can be further supported by the interest in traditional and regional foods that has become a new consumer trend in food markets all around the world and by the European legislation that aims to protect agricultural products with geographical indication and designation of origin[36].

CONCLUSIONS

The present study examined selected milk quality parameters of a Mediterranean dairy sheep breed that is usually reared in less favoured, rural and mountainous areas and revealed that the milk composition of the Arta dairy sheep breed is good. In order to support farmers' income the good milk quality should be maintained and other economic activities, like on- farm cheese manufacturing should be undertaken.

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Preliminary genetic variability analysis of the native Garfagnina goats based on microsatellite polymorphism

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SUMMARY

For the development of an appropriate programme for conservation of animal genetic resources, genetic typifying is considered an important preliminary step. In this paper, we have done a preliminary genetic variability analysis of 48 adult Garfagnina goats belonging to a single flock of Tuscany using 12 STR markers (MAF065, SRCRSP05, INRA023, McM527, CSRD247, SRCRSP23, OarFCB20, TGLA53, INRA005, INRA063, ETH10, ILSTS87) some of which belonged to a markers panel validated by the International Society of Animal Genetics (ISAG) and others routinely used by the facilities of the Laboratorio di Genetica e Servizi (Associazione Italiana Allevatori, Migliaro, Italy). Garfagnina is an Italian native goat breed registered on the Tuscan regional repertory of genetic resources at risk of extinction and have a total of about 745 animals belonging to 17 flocks. Garfagnina breed is important for livestock biodiversity preservation, being a key animal for specialized cheese market in the Tuscan region.

For each marker the following parameters were computed: number of alleles, effective allele size, observed heterozygosity and polymorphism information content (PIC). Allelic frequencies were estimated by direct counting. To analyze the genetic variability of the population, the following parameters were computed at population level: molecular co-ancestry coefficients (fij), kinship distance (Dk), and inbreeding coefficient (Fi). Moreover, genetic similarities (GS) among all animals were investigated using the Individual Multilocus Genotype. The number of alleles ranged from 3 to 9 (mean 5.92) whereas the expected heterozygosity ranged from 0.48 to 0.83 (mean 0.69). There was a high genetic similarity within the whole population (0.43) showing the great homogeneity of the sampled animals, as confirmed also by the small kinship distance (0.34). However inbreeding coefficient was low (0.32). The results of this research indicate that, despite the fact that animals are considered to belong to the same breeding, the genetic variability of this Garfagnina goat population is acceptable for a population with a reduced numerical value.

KEY WORDS

Genetic variability, STR markers, Garfagnina goats.

INTRODUCTION

Italy has a long history of goats breeding and, despite a dramatic number contraction occurred in the last century, goat farming is still an important reality on the Italian livestock panorama. Many different goats breeds are diffused throughout the entire Italian territory from the green Alpine regions to the dry southern and island ones and they may represent a unique source of genetic diversity. The knowledge of the genetic variability is essential to preserve and exploit biodiversity; the genetic variability of a population can be estimated from genealogical data or using the short tandem repeat (STR) molecular markers both in livestock[1,2,3,4,5] and in pet animals[6,7]. At the molecular level more recently SNPs have also been introduced[8]. In this context, the purpose of this work was that to make a preliminary genetic variability analysis of the native Garfagnina goats based on microsatellite polymorphism. Garfagnina breed is important for livestock biodiversity preservation, being a key animal for specialized cheese market in the Tuscan region. Garfagnina is an Italian native goat population registered on the Tuscan regional repertory of genetic resources at risk of extinction, with about 745 animals belonging to 17 flocks. The origin of this population is still uncertain, even if it seems to derive from crossings between native goats from Alpine Arc and from the Tuscan-Emilian Apennines; local breeders refer that the population was reared for generations for its milk and meat production.

MATERIALS AND METHODS

The study was performed in a Garfagnina goat breed flock consisting of 269 females and 20 males. Age ranged from 2 to 9 years. All animals were registered in the herdbook, but genealogical information was not available. The flock was located in the Garfagnana district (Media Valle del Serchio, Lucca, Italy) and a semi-extensive farming was practiced. The goats grazed during the morning (feed supplements are given mainly over the winter), and were housed overnight, when they received an integration of forage and feed. Flock management was of a family farm type. Milking was practiced twice a day using a trolley milking and the milk was conveyed in refrigerated tanks. Blood samples from the 48 Garfagnina goats, were collected according to the recommendations of the European Council (1986) concerning animal care. Whole blood was collected in Vacutainer tubes with K-EDTA as anticoagulant and stored at −20 °C until genomic DNA was extracted using Qiagen QIAamp DNA blood mini/midi kit (Qiagen, San Diego, CA, USA).

Twelve microsatellites (MAF065, SRCRSP05, INRA023, McM527, CSRD247, SRCRSP23, OarFCB20, TGLA53, INRA005,

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Table 1 - Locus, Dye, range, number of alleles, effective allele size (EfAlSize), observed heterozygosity (Ho) and polymorphism information content (PIC), for the 12 microsatellite loci.

Locus	Dye	Range	Chromosome	N° alleles	Ef.AlSize	Ho	PIC
SRCRSP23	6-FAM	69-111	unknown	9	5,35	0.831	79.31%
OarFCB20	PET	86-118	2	4	2.57	0.611	56.30%
MAF065	VIC	115-151	15	9	5.87	0.830	80.97%
ILSTS87	6-FAM	137-151	28	7	5.38	0.814	78.77%
INRA005	NED	110-126	12	5	2.49	0.599	53.34%
TGLA53	PET	130-160	16	5	3.14	0.681	61.89%
McM527	NED	162-178	5	6	3.58	0.721	67.30%
SRCRSP05	VIC	153-181	21	6	4.17	0.760	72.51%
INRA063	6-FAM	169-179	18	4	1.93	0.482	40.72%
INRA023	VIC	190-220	3	7	2.91	0.656	61.98%
ETH10	NED	198-286	5	3	2.53	0.605	53.60%
CSRD247	6-FAM	211-263	14	6	3.62	0.724	67.31%

INRA063, ETH10, ILSTS87), located in 12 chromosomes and amplified in one multiplex PCR reactions, were investigated. Detailed information of these markers is reported in Table 1. Some STR belonged to a markers panel validated by the International Society of Animal Genetics (ISAG) and others were routinely used by the facilities of the Laboratorio di Genetica e Servizi (Associazione Italiana Allevatori, Migliaro, Italy).

For each marker the following parameters were computed using the Molkin v2.0 program[9]: number of alleles, effective allele size, observed heterozygosity and polymorphism information content (PIC). Allelic frequencies were estimated by direct counting. To analyze the genetic variability of the population, the following parameters were computed at population level by using the MolKin program[9]: molecular co-ancestry coefficients (fij), kinship distance (Dk), and inbreeding coefficient (Fi). The molecular co-ancestry between 2 individuals, i and j (fij), is the probability that two randomly sampled alleles from the same locus in 2 individuals are identical by state (Caballero and Toro, 2002). The molecular co-ancestry of an individual i with itself is self-co-ancestry (si), which is related to the coefficient of inbreeding of an individual i (Fi) by the formula Fi = 2si -1. In turn, the kinship distance (Dk) between 2 individuals i and j is Dk = [(si +sj)/2] - fij[10]. MolKin computes within-breed molecular co-ancestry and Dk by simply averaging the corresponding values for all the within-population pairs of individuals. Moreover, genetic similarities among all animals were investigated by comparing the individual multilocus genotype of each individual. Genetic similarity is defined as P=A/2L, where P is the proportion of common alleles (A) in relation to the 2L possibilities (L=number of considered loci). The similarities between each pair of individuals were then averaged over the whole population.

RESULTS

The results of the microsatellite analysis in term of number of alleles observed, alleles size, PIC and observed heterozygosity of the analyzed Garfagnina goat population are summarized in Table 1. In total, 71 alleles were observed for the 12 microsatellite loci analyzed. All 12 microsatellite markers resulted to be polymorphic. Table 2 reports the percentage of each of the most frequent alleles (>10%) for each marker.

The most polymorphic loci were: MAF065 and SRCRSP23 (9 alleles) (Table 1) but the alleles with a frequency higher than 10% were respectively 3 and 5 (Table 2); on the contrary the less polymorphic loci were: ETH10 (3 alleles), INRA063 and OarFCB20 (4 alleles). All the alleles of ETH10 marker and all the alleles of OarFCB20 had a frequency higher than 10% (Table 2).

The PIC per locus showed only one marker with values under the 50% (INRA063) and an average value of 64.5% (±12.30). Genetic similarity within the population (GS), the mean molecular co-ancestry (fij), the kinship distance (Dk) and the inbreeding coefficient (Fi) were 0.430, 0.308, 0.304 and 0.318 respectively (Table 3).

Table 2 - Percentage of each of the most frequent alleles (> 10%) for each marker.

Marker	OarFCB20	Marker	ILSTS87
Allele	93 95 97 99	Allele	137 139 141 143 145
Frequency	0.23 0.13 0.53 0.11	Frequency	0.17 0.16 0.26 0.17 0.19
Marker	INRA005	Marker	MAF065
Allele	115 117	Allele	121 131 133
Frequency	0.54 0.31	Frequency	0.15 0.28 0.22
Marker	McM527	Marker	TGLA53
Allele	152 162 164	Allele	134 136 146
Frequency	0.24 0.26 0.39	Frequency	0.36 0.38 0.21
Marker	INRA063	Marker	SRCRSP05
Allele	173 175	Allele	163 171 173 179
Frequency	0.29 0.66	Frequency	0.26 0.10 0.36 0.18
Marker	ETH10	Marker	INRA023
Allele	203 205 207	Allele	197 211 215
Frequency	0.21 0.48 0.31	Frequency	0.15 0.53 0.19
Marker	CSRD247	Marker	SRCRSP23
Allele	228 230 240	Allele	79 91 95 101 105
Frequency	0.30 0.26 0.33	Frequency	0.34 0.14 0.10 0.11 0.14

Table 3 - Within-population diversity.

	N	Average n° of alleles	Ho*	f_{ij}#	F_i‡	D_K§	GS‖
Total	48	5.92±1.881	0.693	0.308	0.318	0.340	0.430±0.103

Ho*: observed heterozygosity. fij#: molecular co-ancestry coefficients.
F_i‡: inbreeding coefficient. D_K§: kinship distance. GS‖: genetic similarity.

DISCUSSION AND CONCLUSION

There is dearth of published reports on the genetic variability and on the number of alleles, their size and frequencies for microsatellite loci in goats. Although a comparison with other breeds can be biased due to the different marker sets used by different authors, it may be noted how the mean number of alleles per locus was slightly lower than that reported by Ramamoorthi et al.[11] on the Barbari goats, and by Sechi et al.[12] on three Sardinian goat populations, but similar to what observed in Orobica and Girgentana goats by Negrini et al.[4]. Orobica and Girgentana exhibited small genetic variability and therefore evidence of a recent bottleneck. However, our animals derived from a single flock.

The PIC estimated in the present study is comparable with that obtained in other goat breeds, such as Saanen[4] . The PIC was originally introduced by Botstein et al.[13]. It refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency and has been proved to be a general measure of how informative a marker is; the higher is the PIC value, the more informative a marker is. In the present study, MAF065 and SCRRSP23 and ILSTS87 microsatellites appeared the most informative, whereas INRA063 the less informative.

Ten of the 12 markers considered in this research were used also by Sechi et al.[12] for the study of the genetic variability in Maltese, Sardo autochthonous goats and their mixed blood population, and by Negrini et al.[4] who analyzed the genetic structure of eight Italian goat breeds (Camosciata delle Alpi, Valodostana, Bionda dell'Adamello, Orobica, Grigia Molisana, Girgentana, Argentata dell'Etna, and Sarda).

Genetic similarity within our population is higher than reported in other species such as cattle[3] and sheep[2,5], but lower than that reported in two Italian autochthonous donkey genetic types[14] and in Bracco Italiano dog breed[6]. With the exception of the values reported in Amiata and Viterbese donkey breeds [14] and in Bracco Italiano dog breed[6], the values observed in our study regarding the mean molecular co-ancestry was clearly greater than those reported in sheep[2,5] and in cattle[3]. Conversely, the kinship distance was smaller than that reported in literature.

In a previous study[15] the same animals were also genotyped using the Illumina GoatSNP60 BeadChip (Illumina Inc., San Diego, CA), containing 53,347 SNPs, designed by the International Goat Genome Consortium (IGGC). Selected SNPs were used to verify the population structure of this goat population. A matrix of genomic relationships was calculated and a matrix of genetic distances was built. Three main clusters (Group 1: 33 animals; Group 2: 6 animals; Group 3: 9 animals) has been identified. Even if it was not possible to trace back the ancestors of any individual, we postulated that each cluster refers to daughters of related individuals. This explains the high relationship between the animals observed by the microsatellites analysis.

In conclusion, the analysis performed with the use of 12 microsatellite markers showed that despite all goats originated from the same flock and are presumably subdivided into 3 groups with different ascenders, the inbreeding was lower than that reported in all the paper previously mentioned and the genetic variability was acceptable for a population with a reduced effective numerical value. Future work may include replication of this study with a larger number of animals belonging to different flocks.

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Autologous prosthesis for the surgery of two simultaneous hernias in a calf

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SUMMARY

The authors propose a rare case of double hernia in a female, red freisian calf, seventeen months old. Hernia that occurs most frequently in calf is related to hereditary factors or mainly traumatic causes. The clinical examination of the lesion led to detect two similar lesions, round and smooth, not-hot and not-sore, the first localized to the umbilical region, and the second, more caudal and larger, in a paramedian position. Both lesions were palpable in the abdomen through regular continuous and non-communicating solutions. After some basic analysis, the calf received general anaesthesia and underwent standard surgery, but with the application of autologous prosthetic material, seen the location and peculiarity of the lesion. After two months the calf was sound and all her functions restored. Double hernia requires to strengthen the suture with prosthetic material. A flap of the hernial bag wall has been used as prosthetic material, since is strong and well-vascularized, provides excellent tissue compatibility and guarantees that, thanks to constant blood flow, there is always the presence of cellular reaction elements with the formation of a stiff fibrin cloth and a healthy healing tissue. After the surgical procedures, the calf was awakened regularly. At a first check the day after the surgical procedure, the animal regularly resumed eating and drinking. After two months, the patient's follow-up confirmed the complete healing of the lesions, with functional recovery of the herniated organs and the general condition of the subject. The use of an autologous prosthesis comes from a twofold consideration: the great stress that tissues are subjected to an animal with a double hernia, requires the application of a reinforcement that remains stable for at least 15 days in the postoperative. In addition, the autologous prosthesis, made up of well-vascular tissue, has excellent compatibility between tissues. Such features favor the formation of new granulation and fibrin tissues in a short time, thus reducing healing times.

KEY WORDS

Calf, hernia, autologous prosthesis, laparocele.

INTRODUCTION

Hernia that occurs most frequently in calf is related to hereditary factors or mainly traumatic causes. These are mainly external hernias affecting the abdominal region, the inguinal region and the pelvic region[1,2,4]. The hernia content may sometimes reach a considerable size, with the loss of the possibility of reduction[2,4,6]. In acute cases, as in congenital cases, the lesion begins without significant systemic involvement. In chronic cases, or ones complicated by consecutive or inflammatory lesions (umbilical hernia above all), the anamnesis reports a progressive deterioration of the organic conditions, with decrease in growth and / or milk production[1,2,4]. The lesion usually detects a variable volume swelling, generally rounded and regular, not hot and not painful. An accurate compression of the hernia, in most cases, allows the disappearance of the swelling and perceiving the rupture door[1,2,4,9]. In chronic cases, where phenomena of flogosis and sclerosis modify the anatomical relationships between the various structures, it is still possible to palpate the lesion without the animal's painful reactions, but it is impossible to reduce swelling. In complicated cases of secondary injuries, in addition to a general state of general suffering, the lesion is hot, very sore and intractable[6,7,8]. For differential diagnosis, it is sufficient to perform a puncture of the lesion. In some cases, however, the above method is useless: for example, in some chronicized umbilical abscesses, the walls are so thick, often concatenated, and the pus is so dense that the centesis does not provide significant data. In these cases, an ultrasound examination should be performed to obtain verifiable data on the tumor content and, therefore, to establish appropriate therapy[6,7,8].

There are various techniques in the literature that are useful for the treatment of hernia: the size of the lesion should be taken into account[7,9,10]. It should be remembered that subjects with congenital hernia should be excluded from reproduction as it is a transmissible hereditary factor. By leaving behind the conservative techniques described in the literature, it is necessary to state that larger ruptures, as well as the complicated ones, require classical surgical therapy[7,8,9]. If there is a doubt about a poor suture holding, for example in very large animals or if the abdominal walls are altered by the chronicization of the lesion, some authors suggest that a network of resorbable synthetic material be applied to the suture of the rupture door, in order to reinforce the latter[6,9,10]. In this regard, since the value of these alloprostheses is recognized in the success of surgery and in

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the prevention of recurrences, although their costs are high and certainly unrepresentable in the case of income-producing animal surgery, it is thought to replace synthetic prosthetic material with an autologous prosthesis, obtained by creating a vascularized flap from the hernia bag[6,9,10]. This surgical method is intended to confer considerable resistance to erniorraphy and to prevent recurrences, without using synthetic prosthetic materials.

MATERIALS AND METHODS

A seventeen months old female calf, of red-headed Italian, was enrolled in this study. It was visited and operated at the same time as a field clinic, carried out in the province of Ragusa (Sicily).

The objective examination of the lesion allowed to detect a swelling of size 8x7 cm, of round shape and regular, not hot and not painful, localized to the umbilical region, and a second caudal and larger, of the size of 12x9 cm, in a paramedian position, with the same anatomical characteristics as the first one.

The lesion doors had a diameter of 4 cm and 7 cm respectively (Fig. 1). Both swellings were palpable in the abdomen through regular, round, non-communicating lesion doors (Fig. 2). Upon completion of the clinical trial, a diagnosis of double laparocele was formulated. Therefore, it has been programmed for surgery for a few days. The subject was placed on 24-hour pre-operative fasting, while the water was suspended from the previous evening. Before the operation, the animal was subjected to a further accurate clinical and anesthesiological examination. Haematological and haematochemicalanalysis have been performed that have given values to the standard.

The patient was anesthetized with xylazine hydrochloride, equal to 0.2 mgkg-1, followed by an intravenous ketamine hydrochloride infusion, at a dose of 5 mgkg-1, then tramadol 1 mgkg-1, administered at the end of the dorsal decubitus[3,5]. Throughout the duration of the intervention, intravenous access was maintained by NaCl 0.9% perfusion. The surgery site, that is skin, subcutaneous tissue, surrounding tissues and hernial door, has been infiltrated, with full thickness, with lidocaine at 2%[3,5].

Once reached an appropriate stage of anesthesia and analgesia, after having prepared the surgical field according to surgical art, the skin was incised and then, with the scissors, bluntly, the surrounding tissues, to isolate the hernia bag. This was incised, thus highlighting the hinged door and its contents.

The dislocated organs were appropriately repositioned through the hinged door. After having gunned the sclerotic edge of the door, traditional erniorraphy was performed, with detachable nodous points and monofilament n. 2 absorbable wire. The remaining hernia bag was shaped and used, by attachment and fixation, to reinforce the suture, with detached nipple stitches, also with resinable monofilament n. 2 (Fig. 3). It was then sutured the subcutaneous plane with detached sutures and the skin.

The interventions carried out on the two lesions overlap as surgical procedures: first, on the onphalocele and then on the other lesion, with two separate operating fields but with the same surgical technique.

Figure 1 - Seventeen months old female calf. It is possible to see the double swelling located at the level of the caudal abdominal wall.

Figure 2 - The openings of the double hernias. With the simple finger pressure, it is possible to replace the herniated organs.

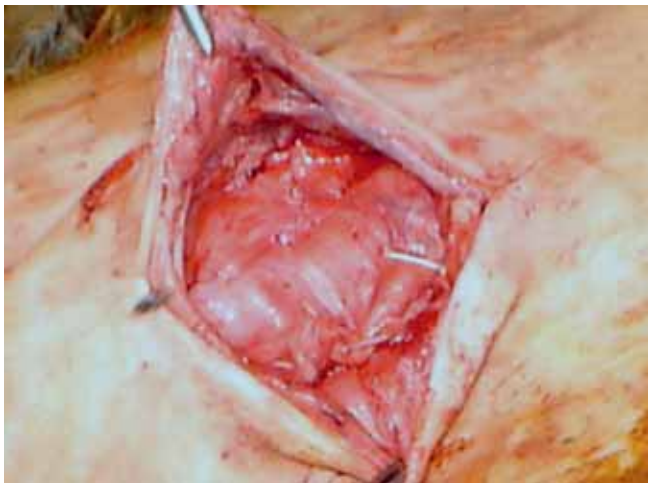

Figure 3 - View of the surgical field, with the autologous flap already sutured in place.

RESULTS

Immediately after the surgical procedures, the calf was awakened regularly. At a first check the day after the surgical procedure, the animal regularly resumed eating and

drinking. After two months, the patient's follow-up confirmed the complete healing of the lesions, with functional recovery of the herniated organs and the general condition of the subject.

DISCUSSIONS AND CONCLUSIONS

Although the external appearance led immediately to think of two congenital hernias, intraoperatively, by the evaluation of the rupture's rings ring, it was suggested that paramedic hernia could be of traumatic nature, occurring during childbirth or immediately postpartum. In fact, this second hinged door was presented with jagged and uneven margins, unlike the umbilical, regular, and fibrous margins.

The idea of using an autologous prosthesis came from a twofold consideration: in the meantime, the great stress to which the tissues are subjected, especially in an animal with a double hernia, which requires the application of a reinforcement that remains stable for at least fifteen days in postoperative; in addition, the hernia bag, strong and well-vascularized, provides excellent tissue compatibility that, thanks to constant blood flow, guarantees the presence of cellular defense and reactive elements with the formation of a stiff fibrin layer.

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UNA FINESTRA SULLE AZIENDE

Supportare la salute intestinale dei vitelli durante i primi freddi? Oggi si può!

Alltech Gut Health Management è il programma Alltech nato per fornire soluzioni naturali per il miglioramento della salute intestinale. Animali sani mangeranno e produrranno in modo più efficiente, al massimo del loro potenziale. Alltech Gut Health Management si focalizza sul miglioramento delle performance animali promuovendo la microflora intestinale, un sistema immunitario sano e massimizzando la crescita e l'efficienza alimentare.

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Problematiche gastroenteriche con episodi diarroici possono essere legate a turbe digestive, infezioni parassitarie, batteriche e/o virali. Alltech propone soluzioni naturali per contrastare le forme enteriche acute alimentari e infettive che si possono presentare.

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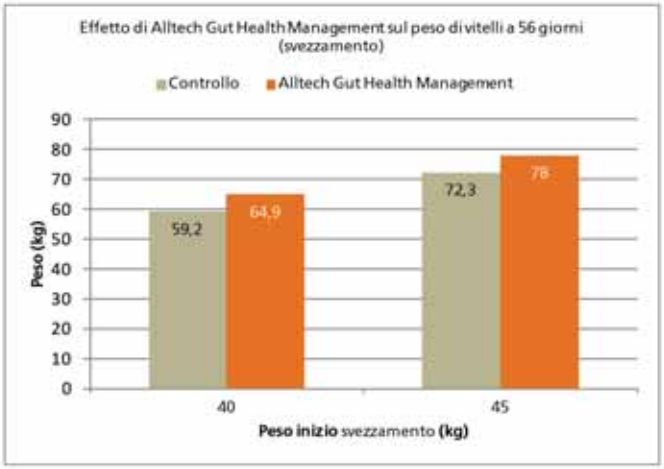

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La prevenzione delle malattie mediante la vaccinazione degli animali inoltre riduce la necessità di utilizzare gli antibiotici, creando quindi un circolo virtuoso per l'allevamento.

Le vaccinazioni, nell'ambito di un programma di prevenzione globale, possono contribuire notevolmente alla riduzione della gravità e della frequenza delle malattie infettive, e dunque ridurre la necessità di utilizzare alcuni farmaci, quali gli antibiotici, per il trattamento delle infezioni.

Nonostante le evidenze che dimostrano i benefici della vaccinazione come parte integrante di un piano preventivo, gli allevatori spesso attendono prima di vaccinare, fino alla comparsa della malattia. L'obiettivo dell'iniziativa **"Time to Vaccinate"** è quello di spostare l'attenzione degli allevatori dal rendere gli animali sani al mantenerli sani.

Aumentare il tasso di vaccinazione e quindi l'immunità contro i patogeni cui vitelli e bovini adulti sono generalmente esposti, consente di migliorare la produttività, la salute generale ed il benessere degli animali.

"I vaccini sono strumenti indispensabili per prevenire malattie infettive potenzialmente pericolose, per mantenere il benessere degli animali ed ottimizzare la produzione", spiega la dr.ssa Catharina Berge, del Berge Veterinarian Consulting, BVBA. "Oggi, solo il 20% circa degli allevatori europei vaccina per prevenire alcune delle malattie più frequenti che colpiscono i loro animali. Se incrementassero il tasso di vaccinazione l'industria potrebbe raggiungere obiettivi di produzione più elevati, con un forte ritorno sugli investimenti e una potenziale eradicazione delle malattie."

Il programma, che esorta gli allevatori a condividere le proprie esperienze sulla gestione delle patologie, si svilupperà nei vari Paesi durante tutto l'anno. Testimonianze, corsi di formazione e connessioni in tempo reale suggeriranno a molti allevatori un nuovo modo di affrontare la realtà, dando loro l'opportunità di apprendere da colleghi che hanno già sperimentato come mantenere le mandrie produttive.

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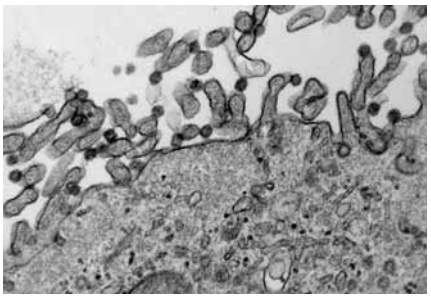


Influenza D: un'infezione emergente nei suini italiani

Rilevanza epidemiologica del patogeno in aumento, secondo uno studio condotto nella Valle del Po

Il virus dell'Influenza D (IDV), nuovo membro della famiglia Orthomyxoviridae, è stato segnalato per la prima volta nei suini in Oklahoma nel 2011 e successivamente riscontrato nei bovini in Nordamerica ed Eurasia. Al fine di indagare la circolazione di IDV nei suini iitaliani, nel periodo tra giugno 2015 e maggio 2016 si effettuavano test biomolecolari e virologici su 845 campioni clinici prelevati da 448 allevamenti suini affetti da problemi respiratori e localizzati nella valle del Po. Si effettuavano test sierologici su 3698 sieri suini, includendo sieri prelevati nel 2009, così come campioni prelevati nel 2015 nella stessa regione. Si identificava il genoma virale in 21 campioni (2,3%) di 9 allevamenti (2%), mentre il virus veniva isolato con successo da tre campioni. Le analisi genetiche evidenziavano che gli IDV dei suini italiani erano strettamente correlati al cluster D/swine/Oklahoma/1334/2011. I sieri prelevati nel 2015 mostravano un'elevata prevalenza di titoli anticorpali IDV (11,7%), mentre i sieri del 2009 mostravano tassi di positività significativamente inferiori (0,6%).
I risultati indicano che la rilevanza epidemiologica del patogeno è in aumento e che sono necessari ulteriori indagini per comprendere la patogenesi, l'epidemiologia e il possibile potenziale zoonosico di questo virus emergente, concludono gli autori.

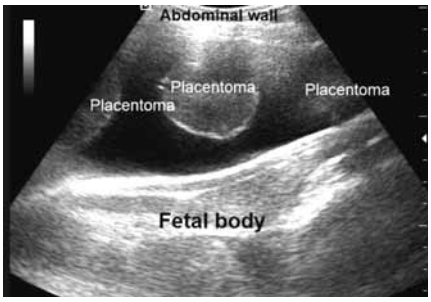
"Influenza D in Italy: towards a better understanding of an emerging viral infection in swine." Foni E, Chiapponi C, Baioni L, Zanni I, Merenda M, Rosignoli C, Kyriakis CS, Luini MV, Mandola ML, Bolzoni L, Nigrelli AD, Faccini S. Sci Rep. 2017 Sep 15; 7 (1): 11660.

Patologie neurologiche dei ruminanti: studio retrospettivo di coorte

Paresi spastica, osteomielite vertebrale e listeriosi le diagnosi più frequenti nel corso di 10 anni

Nel corso di 10 anni (2006-2016), presso l'Università di Glascow in Scozia si osservavano 96 ruminanti con segni neurologici, costituenti il 5,4% di tutte le visite effettuate. Erano descritti 47 diversi segni neurologici di presentazione e il 79% dei pazienti presentava anomalie di andatura. Tutti i casi con anomalie in più di 4 su 10 categorie neurologiche decedevano o erano soppressi per motivi di benessere. I vitelli avevano una probabilità significativamente maggiore di presentare patologie neurologiche rispetto ai bovini adulti. Le lesioni erano più spesso localizzate al midollo spinale nel 47% delle pecore (16), al sistema nervoso periferico nel 45% dei bovini (28) e a livello cerebrale nel 41% della popolazione complessiva (39). Le cause più comuni delle patologie neurologiche osservate erano infettive o infiammatorie (28%, 27). Una diagnosi definitiva era raggiunta nell'84% (81) dei pazienti. Quando era disponibile un esame autoptico, esso produceva una diagnosi nel 70% (50) dei casi e contraddiceva la diagnosi clinica nel 38% dei casi (26).
Le diagnosi più frequenti nei ruminanti nel corso di 10 anni erano paresi spastica, osteomielite vertebrale e listeriosi.

"Ruminant neurological disease: a retrospective cohort study". Giles L, et al. Vet Rec. 2017 Sep 5.[Epub ahead of print].



L'utero bovino gravido non è un ambiente sterile

Batteri presenti nel 90% circa dei campioni endometriali e placentari di uno studio, non associati a infiammazione

Nella maggior parte delle bovine si verifica un'invasione batterica dell'utero durante il periodo postpartum ma si ritiene generalmente che tali batteri siano eliminati prima della gravidanza successiva. L'utero gravido è quindi stato fino ad oggi considerato un ambiente sterile, tuttavia questo assunto è stato ora messo in discussione da recenti studi nell'uomo che indicano che è possibile la presenza di batteri nella placenta della gravidanza a termine senza associazione con l'aborto.
Uno studio ha indagato se fossero presenti batteri nell'utero gravido bovino. Si prelevavano campioni di endometrio inter-caruncolare e di placentoma da 43 bovine gravide al macello, sottoponendoli a esame istopatologico, ibridazione fluorescente in situ e sequenziamento massivo parallelo. Si osservavano batteri nei tessuti del 90,7% (39/43) delle bovine mediante ibridazione fluorescente in situ. Fusobacterium necrophorum, Porphyromonas levii e Trueperella pyogenes erano localizzati all'interno dell'endometrio, sulla superficie endometriale e nello stroma caruncolare, ma la loro presenza non era associata a infiammazione. I dati del sequenziamento masssivo parallelo del gene del 16S rRNA di un sottogruppo di 15 bovine indicavano che i batteri più abbondanti erano della famiglia Porphyromonadaceae, seguita da Ruminococcaceae e Lachnospiraceae. I risultati indicano che l'utero bovino non è un ambiente sterile durante la gravidanza, come precedentemente ritenuto, e che la bovina può condurre la gravidanza anche in presenza in utero di alcuni batteri potenzialmente patogeni, concludono gli autori.

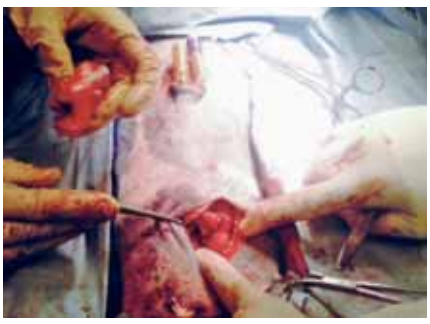
"Presence of bacteria in the endometrium and placentomes of pregnant cows". Cecilia Christensen Karstrup. Theriogenology. September 1, 2017. Volume 99, Pages 41-47.

Anastomosi vescicoprepuziale per l'urolitiasi ostruttiva del becco

Una procedura chirurgica praticabile per i casi ricorrenti, con esito clinico favorevole in uno studio preliminare

Uno studio descrive una nuova tecnica chirurgica per la correzione dell'urolitiasi ostruttiva ricorrente nel becco. Si rivedevano le cartelle cliniche di 4 becchi castrati sottoposti ad anastomosi vescicoprepuziale (VPA) per il trattamento di questa condizione. Tutti gli animali erano stati sottoposti ad almeno una procedura chirurgica (mediana: 2,5; range: 2-4) per la correzione dell'urolitiasi, prima di essere sottoposti a VPA. Le complicazioni postoperatorie includevano la rimozione prematura del catetere dalla vescica (1 soggetto), cistite batterica (2) e formazioni di ascessi (1). In un soggetto si verificava la stenosi dell'anastomosi 3 mesi dopo la procedura iniziale e veniva sottoposto a una seconda VPA; un soggetto decedeva 7 mesi dopo la chirurgia a causa di una grave idronefrosi acuta e insufficienza renale.
La sopravvivenza a lungo termine ≥12 mesi era buona, con 3/4 animali (75%) o 3/5 procedure VPA (60%) con flusso urinario non ostruito a 12 mesi.
L'anastomosi vescicoprepuziale è una procedura chirurgica praticabile per la correzione dell'urolitiasi ostruttiva ricorrente nel becco e può determinare un esito clinico favorevole. Sono auspicabili ulteriori studi su più ampie popolazioni caprine per la valutazione della idoneità della tecnica in questa condizione, concludono gli autori.

"Vesicopreputial anastomosis for the treatment of obstructive urolithiasis in goats." Elizabeth Erin Cypher et al. Vet Surg. February 2017; 46 (2): 281-288.



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