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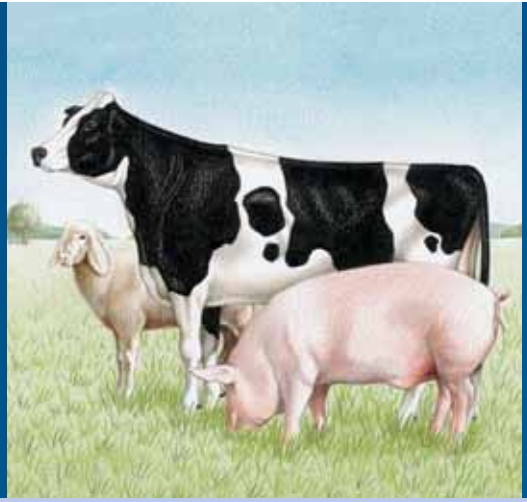

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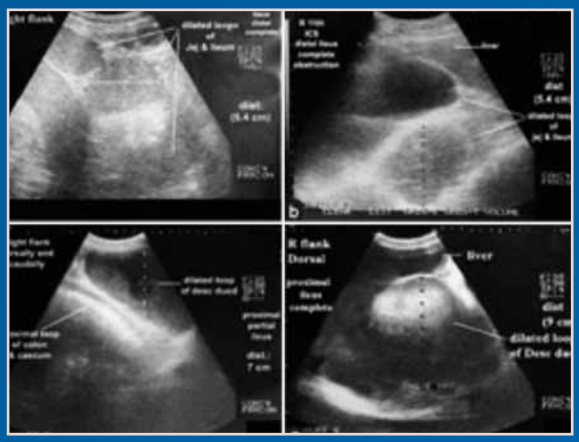
ARTICOLI ORIGINALI

BOVINI

SUINI



CASE REPORTS

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SOMMARIO

ARTICOLI ORIGINALI

CASE REPORTS

RUBRICHE

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. **Modalità di dispensazione:** Da vendersi dietro presentazione di ricetta medico veterinaria in triplice copia non ripetibile.

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Risposta anticorpale sieroneutralizzante e verso le proteine non strutturali NS2-3 indotta da un vaccino inattivato BVD: applicazione pratica in campo

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RIASSUNTO

La gestione del virus della Diarrea Virale Bovina (BVDV) negli allevamenti di bovini da latte risulta complessa e deve generalmente contemplare il ricorso ad un efficace protocollo vaccinale associato alla ricerca ed eliminazione dei soggetti persistentemente infetti (PI), al periodico monitoraggio sierologico e a procedure di biosicurezza. Scopo della presente indagine è quello di valutare l'entità e l'evoluzione nel tempo della risposta anticorpale evocata da un vaccino inattivato del commercio verso BVDV (Bovilis® BVD), con particolare riferimento alla risposta verso le proteine non strutturali NS2-3. Sono stati condotti due studi, il primo volto a valutare l'entità e l'evoluzione nel tempo della risposta anticorpale evocata dal vaccino inattivato Bovilis® BVD verso BVDV, il secondo, per valutare la presenza e l'entità della circolazione di BVDV in mandrie vaccinate con Bovilis® BVD da tempo variabile. I risultati ottenuti confermano l'attività immunizzante sieroneutralizzante evocata dal vaccino dopo vaccinazione di base e richiami effettuati su base semestrale. L'assenza di una risposta anticorpale verso le proteine non strutturali NS2-3, rilevata dopo quattro interventi vaccinali, depone favorevolmente circa la possibilità di discriminare la risposta immunizzante indotta dal vaccino inattivato in oggetto da quella evocata dall'infezione di campo nel contesto del quadro complessivo della mandria. La valutazione del grado di positivizzazione alle proteine NS2-3 in un gruppo di giovani soggetti può fornire al medico veterinario un'indicazione circa la probabile circolazione di BVDV e l'assenza di soggetti PI in mandrie sottoposte da tempo variabile a vaccinazione con vaccino inattivato.

PAROLE CHIAVE

BVDV, NS2-3, vaccino inattivato, bovino da latte.

INTRODUZIONE

Il virus della Diarrea Virale Bovina (BVDV) è attualmente considerato uno dei più importanti agenti patogeni della specie bovina in grado di provocare gravi perdite economiche per il settore a livello mondiale[1-3]. Gli effetti che tale patogeno provoca in una mandria di bovine da latte sono molteplici e tra essi si assiste generalmente a riduzione della produzione lattea, aumento della probabilità di aborto e peggioramento delle performance riproduttive, ritardi di crescita, aumento della suscettibilità degli animali ad altre patologie, aumento della quota di rimonta e della mortalità tra i giovani soggetti[4].

Le differenti manifestazioni cliniche associate all'infezione da BVDV, nonché la loro gravità, sono connesse alle caratteristiche proprie dei differenti genotipi e biotipi che caratterizzano l'agente patogeno. Il BVDV è un Pestivirus appartenente alla famiglia *Flaviviridae*, che comprende il virus della peste suina classica, il virus della border disease (BDV) e due distinti genotipi di BVDV, BVDV tipo 1 e BVDV tipo 2[5]. Il genoma del BVDV è costituito da RNA a singolo filamento e polarità positiva, con un'estensione di 12,3 kb, contenente un solo "open reading frame" fiancheggiato da due "untraslatedregions" (UTR), denominate 5' UTR e 3' UTR. A seguito del processo di traduzione virale si formano proteine strutturali e non strutturali. Tra le proteine strutturali, associate al virione maturo, sono comprese una proteina capsidica (C) e tre glicoproteine dell'envelope (E^{RNS}, E1, E2). La glicoproteina E2 (gp53) veicola gli antigeni maggiormente coinvolti nella risposta sieroneutralizzante; anche la glicoproteina E^{RNS} induce anticorpi in grado di neutralizzare l'azione virale. Tra le proteine non strutturali, comunque coinvolte nel processo di replicazione virale, ricoprono un ruolo importante, soprattutto nella valutazione della risposta anticorpale ai fini diagnostici, le proteine NS2 e NS3, la prima dotata di attività ricombinogena e la seconda di attività enzimatiche essenziali alla replicazione del virus. Il BVDV è inoltre caratterizzato dall'esistenza di un biotipo citopatico ed uno non citopatico, che sono differenziati in base al loro comportamento in coltura su tessuti cellulari e cioè all'assenza o presenza di un visibile effetto citopatico[6]. Entrambi i biotipi possono essere isolati da bovini

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colpiti da Malattia delle Mucose, ma solo il biotipo non citopatico può essere reisolato da animali cronicamente infetti[7]. Le proteine NS2 e NS3 risultano ben distinte nel caso del biotipo BVDV citopatico, mentre costituiscono un complesso proteico unico, denominato NS2-3 nel caso del BVDV non citopatico.
La vaccinazione è un ottimo strumento per il controllo della circolazione del patogeno e viene adottata, secondo schemi differenti ed in base a particolari riscontri diagnostici, anche in modo ufficiale da alcune nazioni nel tentativo di eradicare la patologia[8-11]. Il controllo e l'eradicazione sono però attività rese difficoltose da differenti aspetti, quali i metodi di trasmissione dell'infezione e le caratteristiche del virus, che limitano in alcune situazioni l'efficacia del protocollo diagnostico e vaccinale. Il virus infatti è trasmissibile sia per via verticale che orizzontale, sia a seguito di contatto diretto che indirettamente attraverso attrezzature contaminate. Quando un animale suscettibile si infetta per via orizzontale si trova in una condizione di viremia transitoria ed elimina una bassa quantità di virus per un limitato periodo di tempo prima di diventare protetto da nuove infezioni di BVDV[12-14]. L'aspetto però più caratterizzante il BVDV è la trasmissione verticale che può portare alla nascita di un soggetto immunotollerante o persistentemente infetto (PI). Il soggetto PI elimina per tutto il corso della sua vita, una grande quantità di virus[14]. La diffusione del virus all'interno della mandria è fortemente promossa dall'introduzione di nuovi soggetti, ma anche dalle strutture e dall'organizzazione degli allevamenti in cui generalmente gli animali sono suddivisi in sottogruppi in base ad età o stato fisiologico, ma tra cui avviene un continuo rimescolamento[15].
Oltre agli aspetti epidemiologici, il controllo della BVDV è influenzato dalle caratteristiche del patogeno e dalla possibilità di effettuare uno screening efficace su cui poter pianificare un intervento vaccinale. Gli anticorpi verso le glicoproteine di un ceppo virale, infatti, non neutralizzano necessariamente altri ceppi virali. Le proteine non strutturali del virus presentano un livello inferiore di variabilità, ed in particolare la proteina non strutturale NS2-3 risulta essere conservata e comune ai vari ceppi virali. La ricerca degli anticorpi verso le proteine non strutturali NS2-3 può essere dunque un valido aiuto nel monitoraggio della sieroprevalenza per BVDV in corso di vaccinazione. Tra i prodotti presenti in commercio, Bovilis® BVD è un vaccino inattivato che contiene un ceppo citopatico del virus della BVD. Il vaccino si presenta come una sospensione acquosa per iniezione intramuscolare adiuvantata con idrossido di alluminio. Alcuni autori[16-19] hanno evidenziato che Bovilis® BVD non è in grado di evocare una risposta significativa verso le proteine NS2-3, dopo vaccinazione di base seguita da un richiamo effettuato dopo 3-4 settimane.
Lo scopo della presente indagine è stato quello di valutare l'impiego di Bovilis® BVD nel monitoraggio e nel controllo della Diarrea Virale Bovina in allevamenti di bovine da latte. Per tale scopo sono stati condotti due studi, il primo (Studio A) volto a valutare l'entità e l'evoluzione nel tempo della risposta anticorpale evocata dal vaccino inattivato Bovilis® BVD verso BVDV, con particolare riferimento alla risposta verso le proteine non strutturali NS2-3. Il secondo studio (Studio B) aveva invece lo scopo di valutare la presenza e l'entità della circolazione di BVDV in mandrie vaccinate con Bovilis® BVD da tempo variabile.

MATERIALI E METODI

Studio A

Animali e gruppi sperimentali

Sono state incluse nello studio 23 manze di razza Frisona Italiana di età compresa tra i 6 e 18 mesi di vita animali. Lo studio è stato condotto presso un allevamento commerciale di bovini da latte che per caratteristiche strutturali e livello gestionale è stato considerato ben rappresentativo della realtà zootecnica italiana. L'azienda allevava circa 140 capi in lattazione, 25 animali nella fase di asciutta e 150 giovani soggetti da rimonta di differenti età. Il management aziendale prevedeva l'allevamento dei vitelli in gabbiette singole e il successivo svezzamento in box multipli da 8 soggetti. Fino all'entrata in produzione, gli animali erano allevati in box caratterizzati da un numero variabile di soggetti ma della medesima età. Tutti i soggetti erano sieronegativi verso gli anticorpi BVD (sia sieroneutralizzanti che diretti verso le proteine non strutturali) al momento dell'inizio dello studio. L'assenza di un'attiva circolazione virale in allevamento è stata valutata attraverso la negatività di tutti i soggetti di età inferiore ai 30 mesi di vita verso le proteine NS2-3 e attraverso l'assenza di animali viremici nel gruppo dei soggetti in lattazione eseguita mediante PCR per BVD sul latte di massa.
Gli animali sono stati suddivisi in modo randomizzato in due gruppi: trattamento (14 animali) e controllo (9 animali). Gli animali del gruppo di trattamento sono stati vaccinati con il vaccino Bovilis® BVD (MSD Animal Health) per via intramuscolare (2 ml x capo), due volte a distanza di 28 giorni. Questi animali sono stati sottoposti a ulteriori vaccinazioni di richiamo dopo 6 e 12 mesi dalla vaccinazione di base. In totale gli animali sono stati sottoposti a quattro interventi immunizzanti. Gli animali del gruppo di controllo non sono stati sottoposti ad alcun intervento vaccinale

Parametri indagati

Per la valutazione della risposta anticorpale, tutti i soggetti sono stati sottoposti a prelievo ematico dalla vena caudale all'inizio della prova (giorno 0; V1) e ai giorni di studio 28 (al momento della vaccinazione di richiamo per il gruppo di trattamento; V2), 56, 119 giorni, 210 giorni (al richiamo semestrale per il gruppo trattamento; V3), 241, 294, 360 (al secondo richiamo semestrale per il gruppo trattamento; V4) e 392. I campioni sono stati conferiti al Laboratorio del Dipartimento di Salute Animale dell'Università di Parma e sono stati sottoposti a verifica del livello degli anticorpi sieroneutralizzanti (SN) e degli anticorpi verso le proteine NS2-3 (IDEXX BVDV p80 Ab Test).

Studio B

Popolazione

Nello Studio B sono state incluse 278 aziende di dimensioni variabili tra i 50 ed i 900 capi in lattazione, dislocate lungo tutto il territorio nazionale. Sebbene caratterizzate da consistenza, strutture e livello gestionale differente, le aziende incluse nell'indagine erano caratterizzate dal medesimo protocollo vaccinale per il controllo del BVDV e nello specifico: vaccinazione di base mediante due inoculazioni di Bovilis® BVD con un intervallo di 4 settimane, a partire da 8 mesi d'età e richiamo periodico con lo stesso prodotto ogni 6 mesi in modo continuativo per tutta la carriera produttiva degli animali. Il protocollo vaccinale veniva adottato in ogni azienda da tempo variabile.

Parametri indagati

Per la valutazione della circolazione di BVDV, nelle 278 stalle prese in esame sono stati sottoposti a prelievo ematico dalla vena caudale un gruppo campione di animali (da10 a 20 capi) di età compresa tra 8 e 16 mesi. I campioni sono stati conferiti presso le sedi territoriali degli Istituti Zooprofilattici Sperimentali delle Venezie e della Lombardia ed Emilia Romagna, il Dipartimento di Salute Animale dell'Università di Parma ed il laboratorio dell'Associazione Provinciale Allevatori di Cuneo. Sebbene in sedi diverse, i campioni sono stati analizzati con la medesima metodica per la rilevazione degli anticorpi verso le proteine NS2-3 (IDEXX BVDV p80 Ab Test).

In base alla percentuale di animali positivi le aziende sono state classificate in 3 gruppi: bassa o assente circolazione virale (≤20% di positivi), media circolazione virale (>20% e ≤60% di positivi), alta circolazione virale (≥60% di positivi) come descritto in Tabella 1.

RISULTATI E DISCUSSIONI

Studio A

Le condizioni cliniche degli animali dello Studio A sono risultate eccellenti durante il periodo d'indagine. A circa 9 mesi dall'inizio della prova, un soggetto appartenente al gruppo trattamento è deceduto improvvisamente in assenza di sintomatologia clinica evidente. Indagini necroscopiche effettuate tempestivamente hanno consentito di evidenziare un'infezione clostridica che è stata ritenuta la probabile causa della morte del soggetto.

I risultati dei test utilizzati per la rilevazione dell'immunità umorale sieroneutralizzante sono riportati in Figura 1. Durante tutto il periodo di osservazione, gli animali del gruppo di controllo, sono rimasti costantemente negativi per gli anticorpi sieroneutralizzanti ad indicare l'assenza della circolazione virale nella mandria. Per quanto attiene la risposta sieroneutralizzante rilevata nei soggetti sottoposti ad intervento immunizzante, i dati sono dimostrativi di come il vaccino in oggetto abbia evocato una risposta anticorpale specifica. In particolare, la valutazione del titolo geometrico medio (t.g.m.) di gruppo, ha evidenziato un andamento tipico della risposta immunitaria caratterizzata da picchi di sieroconversione nel mese successivo ai vari interventi di richiamo, a testimonianza dell'utilità di effettuare tale pratica con cadenza semestrale.

Nessun animale del gruppo di controllo è risultato siero-positivo verso gli anticorpi NS2-3 durante il corso dell'indagine, ad ulteriore dimostrazione dell'assenza di una circolazione virale attiva all'interno della popolazione. Parimenti, anche nessun animale vaccinato con il vaccino in oggetto ha sviluppato una risposta anticorpale verso NS2-3 anche dopo ripetute vaccinazioni, a conferma dei dati rilevati in studi precedenti[16-19].

Dai risultati dello Studio A, emerge la totale assenza di una risposta anticorpale verso NS2-3 dopo quattro interventi

Tabella 1 - Valutazione della percentuale di positività per NS2-3 e classificazione delle aziende.

% di positività per anticorpi NS2-3	Classificazione delle aziende	Indicazioni
Campioni negativi o positività inferiore al 20%	Bassa o assente circolazione virale	Monitoraggio sierologico annuale e implementare la biosicurezza
Positività compresa tra 20 e 59%	Media circolazione virale ed improbabile presenza di soggetti PI	Monitoraggio sierologico semestrale e implementare la biosicurezza
Positività superiore al 60%	Elevata circolazione virale con forte sospetto di presenza di soggetti PI	Impostare la ricerca dei soggetti PI e implementare la biosicurezza

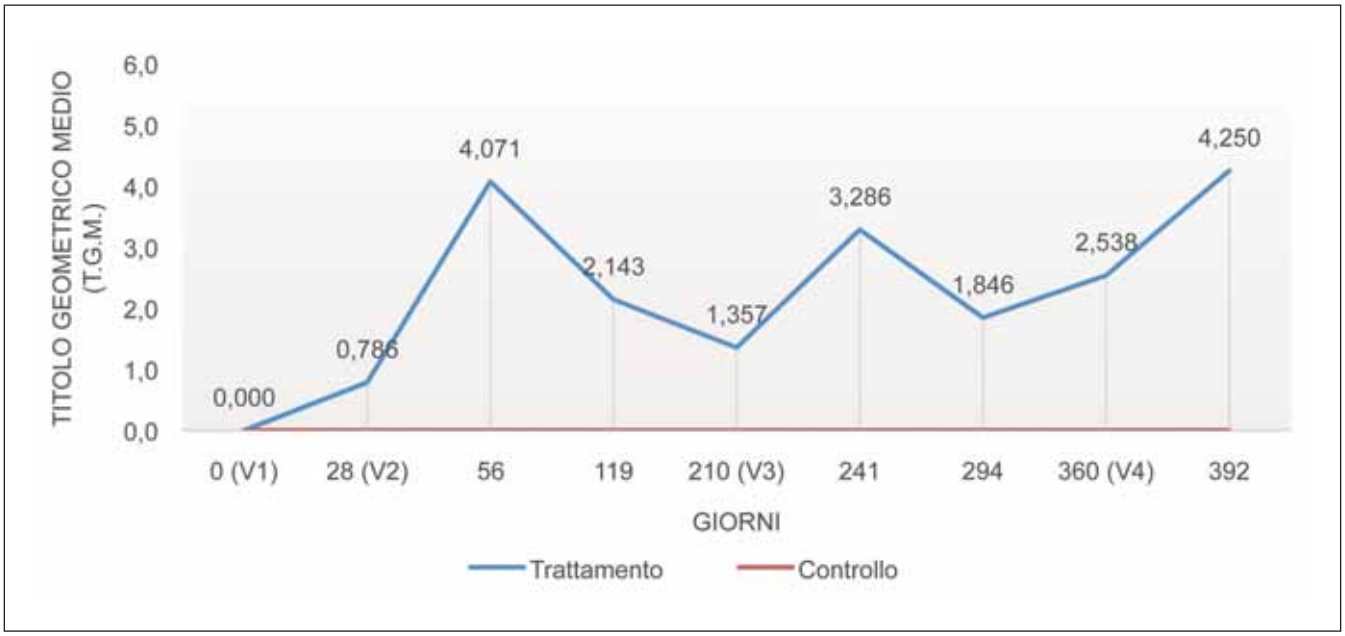

Figura 1 - Risposta anticorpale sieroneutralizzante.

immunizzanti. Con l'impiego in allevamento del vaccino BVDV inattivato oggetto del presente studio sarebbe quindi possibile ipotizzare la valutazione ed il monitoraggio della circolazione attiva del virus (compresa la formulazione del sospetto della presenza di soggetti PI) attraverso la ricerca degli anticorpi verso NS2-3 su un numero significativo di animali di età superiore ai 6-8 mesi di vita (privi di anticorpi materni) ed inferiore ai 30 mesi di vita (sottoposti presumibilmente a meno di 4-5 interventi vaccinali). La ricerca degli anticorpi verso le proteine non strutturali NS2-3 può essere di valido aiuto nel monitoraggio della sieroprevalenza per BVDV in corso di vaccinazione. Questo tipo di approccio è incompatibile con l'utilizzo dei vaccini vivi-attenuati, in quanto questi inducono un'elevata risposta anticorpale verso NS2-3[16], non distinguibile da quella che caratterizza gli animali che contraggono l'infezione di campo. Al contrario, studi condotti con vaccini inattivati hanno mostrato che questi vaccini non inducono, di norma, una significativa risposta verso le proteine NS2-3[17,18,20], e possono essere considerati, almeno in certe condizioni, come vaccini marker[19] atti a distinguere gli animali vaccinati da quelli che hanno contratto l'infezione. Occorre considerare che l'assenza di una risposta anticorpale verso NS2-3 si basa essenzialmente sul comportamento biologico del vaccino inattivato, che prevede assenza di attiva replicazione del ceppo vaccinale, e non sulla delezione genomica del virus o su una specifica tecnica produttiva volta a garantire l'assenza di antigeni NS2-3 nella preparazione vaccinale. Infatti, i vaccini spenti sono allestiti a partire da ceppi virulenti coltivati su tessuto-colture e il surnatante raccolto è successivamente sottoposto ad inattivazione. Non è tuttavia improbabile che quota-parte delle proteine non strutturali, che sono espresse durante la fase di moltiplicazione virale, entri a fare parte, in quantità variabile, del contenuto antigenico del vaccino che è sottoposto ad inattivazione. La quantità residua di proteina NS2-3 può variare nei diversi lotti produttivi, ma comunque non è, di norma, in grado di indurre una significativa risposta verso NS2-3. Si può comunque ipotizzare che dopo ripetute vaccinazioni, si possa rilevare una certa positivizzazione verso NS2-3 legata alla vaccinazione, in assenza d'infezione naturale. Sebbene vi siano buone evidenze della possibile discriminazione, è comunque opportuna una certa cautela nel connotare, in assoluto, i vaccini BVD inattivati di una caratteristica "marker".

Studio B

I risultati delle analisi sierologiche effettuate sul gruppo di animali campione (da 10 a 20 soggetti) di età compresa tra 8 e 16 mesi nelle 278 stalle dislocate sul territorio nazionale, sono riportati in Tabella 2. Circa la metà sono risultate a bassa o assente circolazione virale, il 23% a media circolazione e il 30% ad elevata circolazione. La classificazione nelle 3 fasce è stata proposta dagli autori sulla base dell'epidemiologia del BVDv, in particolare è stata creata un'unica fascia in cui includere sia le stalle negative sia quelle con meno del 20% di animali positivi. Questa scelta è legata al fatto che in questo studio il dato di sieroprevalenza è ottenuto analizzando solo un campione di animali, pertanto non sufficiente per affermare con certezza la completa assenza del virus in allevamento. Inoltre è stato proposto un cut-off a 60% in quanto, sulla base dell'esperienza degli autori, la presenza di immunotolleranti in allevamento porta a positivizzare per NS2-3 almeno il 60% dei soggetti.

Il numero di animali da considerare come campione per la valutazione della sieroprevalenza varia in funzione della dimensione della popolazione e della prevalenza attesa, in questo studio gli autori hanno identificato un range di animali compreso tra 10 e 20 soggetti in quanto sufficiente per rilevare, al 95% di probabilità ed in funzione della popolazione in esame, una prevalenza almeno del 20%. Inoltre un range limitato ad un massimo di 20 soggetti è facilmente applicabile alla realtà di campo.

Gli animali, immunizzati con meno di 5 interventi vaccinali eseguiti con vaccini inattivati, possono essere scelti come animali sentinella al fine di ottemperare ad uno dei 4 pilastri fondamentali su cui si basa il controllo della BVD cioè il monitoraggio. Secondo Houe e colleghi[21] infatti, il controllo della BVD si basa su 4 pilastri fondamentali che sono rappresentati da: vaccinazione, ricerca dei soggetti PI, monitoraggio sierologico e biosicurezza. Sfruttando questo aspetto, la vaccinazione con Bovilis® BVD ha permesso di categorizzare le stalle testate in 3 gruppi in base al numero di giovani animali positivi ad NS2-3. Tale categorizzazione non è certamente discriminante in modo completo in quanto, per aver un quadro più esaustivo occorrerebbe associare alle indagini sierologiche anche l'analisi dei fattori di rischio associati all'ingresso e alla circolazione aziendale del BVDV, oltre ai riscontri clinici e alle performance produttive e riproduttive. Sebbene quindi con le limitazioni procedurali tipiche dell'indagine pratica di campo, un'interpretazione dei dati risulta comunque effettuabile. Il dato più significativo è quello del 47% delle aziende in cui la circolazione virale è risultata assente o ridotta, con meno del 20% di animali positivi agli anticorpi non strutturali. Tale dato è verosimilmente indicativo dell'efficacia dei protocolli di biosicurezza e del piano vaccinale attuato e/o del fatto che il BVDV non stia circolando in azienda. Una totale negatività dei campioni esaminati per anticorpi verso NS2-3, in questo studio rilevata in 99 allevamenti, è fortemente indicativa della probabile assenza di circolazione attiva e di soggetti PI nel gruppo in esame. Una positività inferiore al 20% può essere legata alla presenza di animali che hanno comunque reagito alla vaccinazione o che si sono positivizzati in conseguenza ad una circolazione del virus di scarsa entità e non correlata alla presenza di soggetti PI. In questa situazione è comunque consigliabile effettuare un monitoraggio sierologico a cadenza annuale su animali di età compresa tra 6 e 18 mesi.

Il 23% delle stalle testate aveva un numero di capi positivi compreso tra 21% e 59%. Questa situazione può evidenziare un inizio di circolazione virale conseguente a BVDV recente-

Tabella 2 - Risultati del monitoraggio sierologico verso anticorpi NS2-3.

Aziende analizzate	≤ 20% di positività	21-59% di positività	≥ 60% di positività
278	131 (47%)	64 (23%)	83 (30%)

mente introdotto in azienda, oppure al contrario può testimoniare la progressiva riduzione della presenza del virus nella mandria grazie ai piani sanitari e vaccinali in atto. Questa situazione è di non facile interpretazione, in quanto può indicare la presenza di soggetti PI, ma anche una circolazione virale transitoria in assenza di soggetti PI[22,23]. Al fine di evidenziare precocemente un eventuale incremento di circolazione virale potrebbe essere utile monitorare sierologicamente la mandria a cadenza semestrale testando il gruppo di animali tra 6 e 18 mesi. Infine è stato evidenziato circa un 30% di stalle fortemente positive a BVDV. Le possibili cause di questa situazione possono essere ricondotte a: vaccinazione introdotta da meno di 18-24 mesi, mancata o incompleta ricerca dei soggetti PI, vaccinazione di base non eseguita correttamente, vaccinazione eseguita solo dopo la prima fecondazione, richiami semestrali non rispettati o introduzione in azienda di capi viremici transitori o persistenti. Oltre a tali aspetti, una positività sierologica così elevata è probabilmente connessa alla presenza nel gruppo in esame di uno o più soggetti PI. Infatti, nel corso di studi condotti in precedenza[18], è stata evidenziata, nei gruppi nei quali sono presenti animali immunotolleranti, sieroprevalenze nei confronti delle proteine non strutturali NS2-3 decisamente superiori al 60-70% sia in animali vaccinati sia non vaccinati. In questo caso risulta fondamentale, per il controllo della malattia, affiancare alla vaccinazione la ricerca dei soggetti PI. Un ulteriore punto critico comune della vaccinazione per BVD è rappresentato dalla regolarità di esecuzione degli interventi immunizzanti nei giovani animali. Spesso i vitelli vengono sottoposti alla vaccinazione solamente in corrispondenza dei richiami semestrali di mandria. In questo modo i vitelli più grandi che non vengono vaccinati, verranno immunizzati 6 mesi più tardi, rimanendo così sensibili per più tempo all'azione del BVDV e contribuendo all'amplificazione della circolazione virale in stalla, soprattutto se la prima vaccinazione cade in prossimità della prima fecondazione. Buona norma sarebbe quella di effettuare la vaccinazione di base al termine del periodo di protezione passiva colostrale a circa 6-8 mesi di vita. Tale procedura potrebbe risultare però poco praticabile in campo, in particolare per le aziende di dimensioni medio piccole (<200 capi). Per questo motivo, la vaccinazione di base dei vitelli in 4 momenti durante il corso dell'anno, potrebbe risultare una soluzione comoda ed efficace.

CONCLUSIONI

In conclusione, si ritiene che l'impiego del vaccino in oggetto, oltre a rappresentare un valido strumento nel fornire la copertura immunizzante verso il BVDV, possa inoltre consentire il monitoraggio della circolazione virale attiva mediante una valutazione effettuata non su base individuale, ma su un gruppo di giovani soggetti della mandria. Il dato deve essere interpretato come indicativo non tanto della presenza di soggetti immunotolleranti nell'allevamento, quanto della loro assenza nel momento in cui si rileva negatività sierologica per NS2-3 nella categoria dei giovani animali nella quale è più probabile la presenza dei soggetti PI. Tale metodo di valutazione della possibile circolazione virale in mandrie vaccinate, può essere impiegato dal veterinario aziendale come un rapido ed orientativo metodo di screening iniziale per la gestione del BVDV in allevamento.

Neutralizing and non-structural proteins (NS2-3) antibody response induced by an inactivated BVD vaccine: field application

SUMMARY

Introduction - The management of Bovine Viral Diarrhea Virus (BVDV) in dairy farms is difficult and needs an effective vaccination protocol combined with the search and elimination of persistently infected animals (PI), the periodical serological monitoring and biosecurity procedures. Inactivated BVD vaccines are able to stimulate the antibodies production against the virus without stimulating the production of specific antibodies against non-structural proteins NS2-3 at least within four vaccine interventions. The evaluation of the NS2-3 antibody response could be useful to monitoring the field virus circulation in vaccinated herds.

Aim - Aim of the present work was to evaluate the amount and the evolution during the time of the antibody response elicited by a commercial BVD vaccine (Bovilis® BVD); in particular, as far as the non-structural NS2-3 proteins antibody response is concerned.

Materials and methods - Two studies were performed. Study A involved 23 young heifers of a BVDV free farm, that never received a BVDV vaccination. Animals were divided into two groups: treatments (14 animals) vaccinated 4 times with Bovilis® BVD during one year of investigation and controls (9 animals) without vaccination. Animals have undergone to a periodical evaluation of BVD serum neutralizing antibodies and NS2-3 antibodies. Study B was a survey to monitor the BVDV circulation in 278 dairy farms that used Bovilis® BVD. NS2-3 antibodies were evaluated in a group of young heifers of every farm.

Results and discussion - The obtained results confirmed the virus neutralizing antibody response induced by the vaccine after the basic vaccination and after six-month based boosters. The absence of an antibody response versus the non-structural proteins NS2-3 after four vaccinations suggest that could be possible to differentiate the immune response elicited by the inactivated BVD vaccine from the one elicited by the field infection on a herd base. The titration of NS2-3 antibodies in young heifers can be a useful tool to evaluate the circulation of BVDV in vaccinated herd and the probable absence of PI animals.

Conclusions - The evaluation of the antibody response versus the non-structural proteins NS2-3 in the animals that received no more than four vaccinations can be a good on-farm tool for a preliminary and indicative evaluation of the circulation of BVDV in herds vaccinated with inactivated BVD vaccine.

KEY WORDS

BVD, NS2-3, inactivated vaccine, dairy cattle.

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Behavioural, physiological and productive effects of water unavailability as a form of unpredictable management stressor in dairy cows

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SUMMARY

Introduction - The efficient management of drinking water is an important issue in the dairy farm industry. Lactating dairy cows metabolize large amounts of water, and water deprivation can affect feed intake and milk production. Although accidents which could cause a temporary water unavailability are not unlikely to occur in the dairy cows industry, the effect of such a situation has seldom been scientifically investigated in temperate climates.

Aim - The aim of this study was to assess whether there were behavioural, productive and hormonal effects in dairy cows subjected to 3.5-hour water withdrawal (between 06:00 a.m. and 09:30 a.m.) during winter months in a temperate climate.

Materials and methods - Twelve healthy lactating Holstein dairy cows were studied. During the "changed management" periods, the 3.5-hour water withdrawal was applied either for one (Tuesday, "one-day change condition") or for three consecutive (Tuesday to Thursday, "repeated change") days. The cows were observed by instantaneous scan sampling every 2 minutes for 4.5 hours on Tuesday, Thursday and Saturday. Blood samples were taken at 11.00 on Tuesday and Thursday and cortisol levels analysed by RIA. Data on activity and production were automatically recorded. GLMs, Wilcoxon tests, and ANOVAs were used for analyses.

Results and discussion - Cows moved more and interacted more with structures ($p<0.05$) during water withdrawal, suggesting they were experiencing a certain degree of unfulfilled expectations. However, their overall behaviour was not suggestive of them experiencing a significant stress. No significant differences due to water withdrawal were detected in automatically recorded general activity, which presented marked circadian variations with three peaks (the first between 08:00 and 12:00; the second between 14:00 and 16:00, and the third between 18:00 and 20:00). No significant differences due to water withdrawal were detected in milking parameters and plasma cortisol concentrations.

Conclusions - The 3.5 hour water unavailability during winter months, investigated in the present study, did not alter the behavioural, endocrine and productive parameters in a way suggestive of the cows experiencing significant stress.

KEY WORDS

Behaviour, dairy cows, unpredictable stress, water withdrawal.

INTRODUCTION

The efficient management of drinking water is an important issue in the dairy farm industry. Lactating dairy cows metabolize large amounts of water and this could make them predisposed to be rapidly affected by water deprivation[1]. In cows, sources of water are drinking, feeding, and metabolic (oxidation) processes, whereas water is lost in milk, urine, feces, and various forms of evaporation[1]. Daily water consumption in dairy cows has been shown to range from 14 to 171 kg/cow, with a mean of 82 kg/cow[2]. The main factors influencing water intake in cows are dry matter intake, eating pattern, diet contents of sodium, potassium and nitrogen, ambient and water temperature, humidity, characteristics of the watering equipment, milk production and social factors[1;3].

The importance of the water management issue in the dairy industry is underlined by the fact that water metabolism in cows has been extensively studied in the scientific literature, in most cases with the aim of developing predictive equations of water intake[e.g., 2;3]. Water related issues in dairy cows have also been much investigated in connection with heat stress and/or in extreme climates[4], as the effects of water unavailability on the health, production and welfare of lactating cows can be very severe in extreme heat.

The genetic improvement in the dairy industry in Western counties has led to breeding cows with high milk producing potential, which could be more prone to be affected by water restriction even in less severe conditions. Moreover, in the modern dairy cow industries accidents which could cause a temporary water unavailability are not unlikely to occur, as well as situations in which there is a need to temporarily voluntarily interrupt water availability. It is thus lamentable that there appears to be little research on the possible effects of water unavailability on the welfare of dairy cows in temperate climates.

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The aim of this study was to investigate whether there were behavioural, productive and physiological effects in dairy cows subjected to 3.5 hour water withdrawal, as a form of unpredictable management change.

MATERIALS AND METHODS

The experimental protocol of the study and all procedures were carried out according to the Italian legislation on animal care (Legislative Decree No. 116 of January 27, 1992).
The experiment took place from September to December, which are relatively cool-to-cold months in the area (Latitude = 45° 20' 32 N; Longitude = 11° 57' 53 E) in which the experiment was performed. Temperatures during the study varied between +19° C and -1°C (http://www.scia.isprambiente.it/). No detailed daily/hourly recording of the temperature in the barn was collected during the study as the control group was housed in the same building as the animals undergoing the experimental procedure, thus nullifying the possible bias changes in temperature could have introduced in the results.

Animals and management

Twelve healthy lactating Holstein dairy cows, divided into two groups of six, parity 1 to 5, were studied (Table 1). They belonged to the Padua University experimental farm, which is actually a productive dairy unit.
The cows were loose housed indoors with cubicles in two groups of six with free access to an outdoor pen. Cows in the same experimental group were housed in the same pen. They were individually fed concentrate integration by transponder operated automatic feeders, while TMR (Total Mixed Ration) was given at 07:30 and 13:30. The TMR contained (% on DM basis) grass hay (44.2%), commercial mixed feed for lactating cows (Petrini Group, Italy; 12.3%), dehydrated alfalfa hay (10.2%), dried beet pulp (10.1%), barley meal (9.8%), corn meal (9.8%), commercial calcium esterified fatty acids from palm oil (Maxifat, Consorzio Agrario Padova, Italy; 2.7%), and sodium bicarbonate (0.8%). The composition of the TMR was 51% of DM and contained 13.8% of CP, 43.3% of NDF and 0.88 Milk Feed Units on a DM basis (i.e., 1496 kcal of net energy for lactation, as 1.0 Milk Feed Unit/Kg DM equals to 1700 kcal of Net Energy for lactation, on DM). The cows were milked at 06:45 and 17:00. When no experimental condition was applied, water was available *ad libitum*. This type of management represents a common practice for most of the Italian dairy farms.

Experimental procedure and design

The applied experimental condition to be tested consisted in 3.5-hour water withdrawal (the automatic water-dispensing system was turned off between 06:00 a.m. and 09:30 a.m.). The time of the day in which to withdraw water was chosen in order to include the morning meal and milking times because cows have been shown to drink more around eating and being milked[5].Water withdrawal was applied either for one (Tuesday, "one-day change condition", OC) or for three consecutive (Tuesday to Thursday, "repeated change condition", RC) days, while all other management procedures were kept constant.
In order to control for environmental conditions, the change was never applied to the two groups of cows simultaneously. Thus, each group of cows underwent five different conditions: baseline (BL), "one-day change condition" (OC), "repeated change condition" (RC), control to OC (CT-OC), and control to RC (CT-RC), lasting one week each (Table 2). The order in which the conditions had to be applied was selected by random allocation (a form of restricted randomisation[6]), with one restriction: if one group underwent the "one-day change" first and then the "repeated-change" the opposite had to be true for the other group. The restriction was introduced in order to avoid confounding due to carry over effects. Of course, the baseline condition had to be applied to both groups simultaneously. The random allocation rule was applied as it allows to avoid biases due to arbitrary selection of the order of treatments, in situations is which a true random selection is not suitable due to the need to apply each conditions to all groups and the small number of groups in the experiment. The two conditions were thought to simulate an unexpected one-off event (OC) or a planned maintenance taking place always at the same time and lasting a few days (RC).
A week of preliminary observations preceded the experiment. During the study, the cows were observed by instantaneous scan sampling[7] every 2 minutes. In detail, the two observers took place on a raised platform in front of the cows' pens and waited for 10 minutes in order to habituate the animals to their presence. Then they began collecting data. In order to assess how behaviour changed during the day, 9 half-hour periods (from 07:00 to 07:30, from 07:30 to 08:00, from 09:00 to 09:30, from 09:30 to 10:00, from 10:00 to 10:30, from 11:15 to 11:45, from 11:45 to 12:15, from 13:00 to 13:30 and from 13:30 to 14:00) were selected for observation. These half-hour periods were termed "Hour" in the statistical analyses. These hours were selected to have observation both when the cows were under the effect of the change (i.e., not having access to water) and after the changed situation was removed (i.e., access to water restored). Also practical reasons dictated this choice of timing. The observations were carried out during five weeks on Tuesday, Thursday and Saturday.

Table 1 - Average production characteristics of the two groups of cows.

	Group A	Group B
Parity	2.5 ± 1.52	2.5 ± 1.38
Days in lactation	134 ± 60.29	128 ± 75.74
Production (kg/day)	27.75 ± 3.43	27.75 ± 3.43

Table 2 - Experimental design.

Week	Group 1	Group 2
1	Control to OC (CT-OC)	One-day change condition (OC)
2	Baseline (usual management)	Baseline (usual management)
3	Repeated change condition (RC)	Control to RC (CT-RC)
4	One-day change condition (OC)	Control to OC (CT-OC)
5	Control to RC (CT-RC)	Repeated change condition (RC)

The recorded behavioural categories are listed in Table 3. During the observations, whether the cow was lying or standing was also recorded at each scan. Total standing (all the scans in which the animal was standing irrespectively of which behaviour was expressed, TOT_s) and total lying (all the scans in which the animal was lying irrespectively of which behaviour was expressed, TOT_l) were thus calculated.
Blood samples were taken at 11:00 on Tuesday and Thursday in order to assess cortisol plasma concentrations.
Cortisol levels were analysed using an already validated microplate RIA procedure after diethyl ether extraction and subsequent sample dilution[8].
Data production were automatically recorded every milking session by a dedicated transponder operated automatic system (DeLaval® processor and ALPRO dedicated software). Data concerning general activity were automatically recorded continuously for 24 hours a day using a similar procedure.

Statistical analysis

Instantaneous scan sampling behavioural data - After preliminary exploratory analyses, behavioural data were analysed using G.L.M. procedure, using the software SAS.
The model used in the analysis was:

$$Y_{ijklmn} = \mu + G_i + C(G)_{ij} + W_k + D_l + H_m + GW_{ik} + GD_{il} + WD_{kl} + GWD_{ilk} + GH_{im} + WH_{km} + GWH_{ikm} + DH_{lm} + WDH_{klm} + GDH_{ilm} + GWDH_{iklm} + e_{ijklmn}$$

Where:
μ = general mean;
G_i = fixed effect of Group ($_i$ = 1, 2);
$C(G)_{ij}$ = fixed effect of cow within group ($_j$ = 1,…, 12);
W_k = fixed effect of the week ($_k$ = 1,…5);
D_l = fixed effect of the day of the week ($_l$ = 1,…3);
H_m = fixed effect of the (half)hour of observation in the day ($_m$ = 1,…9);
GW_{ik} = interaction G*W;
GD_{il} = interaction G*D;
WD_{kl} = interaction W*D;
GD_{il} = interaction G*D;
WD_{kl} = interaction W*D;
GWD_{ilk} = interaction G*W*D;
GH_{im} = interaction G*H;
WH_{km} = interaction W*H;
GWH_{ikm} = interaction G*W*H;
DH_{lm} = interaction D*H;
WDH_{klm} = interaction W*D*H;
GDH_{ilm} = interaction G*D*H;
$GWDH_{iklm}$ = interaction G*W*D*H;
e_{ijklmn} = random residual.
A suitable error value was set testing the effect of Group, whereas all other values were tested on the random residual value.
As water withdrawal was applied for some hours in different days depending on week and group, significant short-time effects of the procedure were deemed to appear at the $GWDH_{iklm}$ level.
However, a significant effect of $GWDH_{iklm}$ not necessarily reflects the effect of water withdrawal as it could be due to a

Table 3 - Behavioural categories used for data recording and analysis, their abbreviation and explanation of meaning for each recorded behaviour.

Behavioural Category	Abbreviation	Explanation
Activities towards structures	Act_struct	Licking, nibbling, biting the structures (including the feeding rack/trough and the drinking bowl) and sniffing the structures
Inactivity (standing)	I_s	Standing without performing any apparent specific behavioral pattern
Inactivity (lying)	I_l	Being recumbent without performing any apparent specific behavioral pattern
Movement	Mov	Movements which were not part of other studied behavioral patterns, such as locomotion, lying down, standing up, etc.
Feeding	Fe	Eating TMR standing at feeding rack or eating concentrate at the feeding station
Drinking	D	Ingesting water
Ruminating (standing)	RM_S	Self-explanatory
Ruminating (lying)	RM_L	Self-explanatory
Elimination	Elimin	Micturition, defecation
Self grooming	SGROOM	Licking the surface of one's body, rubbing against structures or other body parts (body care)
Tongue Playing	TP	Performing stereotyped tongue movements
Agonistic activities towards cows	AGON	Butting, displacing another cow
Non-agonistic activities towards other cows	ACT_COW	Licking, nibbling, biting, sniffing, mounting, observing or playing with another cow
Vocalizations	VOC	Self-explanatory. The emission of vocalizations by the animal had to happen while the animal was not performing any other studied behavioral pattern
Coughing	T	Self-explanatory
Passive exploration	EXPL_pass	Sniffing air or observing (head and eyes oriented towards) other cows or people
Other	OTHER	Behavioral patterns not belonging to the any other categories (never observed)
Out of sight	OS	The cow is totally hidden from the observer, or partially hidden, but so that it is impossible to register its behavior

Table 4 - Results of the analysis performed on instantaneous scan sampling behavioural data (only significant results detailed).

	RM_s	RM_l	l_s	l_l	Mov	Fe
Group (G)	NS	NS	NS	NS	NS	NS
Cow	F=2.76; p=0.002	F=6.89; p=0.0001	F=5.89; p=0.001	F=3.33; p=0.0003	NS	F=10.25; p=0.0001
Week (W)	F=4.96; p=0.0006	F=4.15; p=0.0024	F=17.85; p=0.001	F=7.36; p=0.0001	F=6.18; p=0.0001	F=5.48; p=0.0002
Day (D)	F=5.49; p=0.0042	NS	NS	F=3.92; p=0.02	NS	NS
Hour (H)	F=117.2; p=0.0001	F=26.51; p=0.0001	F=22.04; p=0.0001	F=17.64; p=0.0001	F=12.05; p=0.0001	F=153.63; p=0.0001
G*W	NS	NS	F=4.28; p=0.0019	NS	F=4.17; p=0.0023	NS
G*D	NS	NS	NS	NS	F=7.27; p=0.0007	NS
W*D	NS	F=2.06; p=0.037	F=1.95; p=0.0499	F=7.49; p=0.0001	F=3.11; p=0.0017	F=3.11; p=0.0017
G*W*D	F=2.24; p=0.0227	NS	F=3.27; p=0.0011	NS	F=2.24; p=0.0224	NS
G*H	NS	F=2.85; p=0.0038	NS	F=2.84; p=0.0039	NS	F=5.74; p=0.0001
W*H	F=6.2; p=0.0001	NS	F=3.81; p=0.0001	F=1.62; p=0.0161	NS	F=3.09; p=0.0001
G*W*H	NS	NS	F=1.48; p=0.0409	NS	F=1.74; p=0.0068	NS
D*H	NS	F=1.83; p=0.0236	NS	F=2.64; p=0.0004	NS	F=4.74; p=0.0001
W*D*H	F=1.56; p=0.0036	F=2.8; p=0.0001	F=2.07; p=0.0001	F=1.45; p=0.0124	F=1.42; p=0.018	F=3.25; p=0.0001
G*D*H	NS	NS	F=1.75; p=0.0333	NS	F=1.94; p=0.0139	NS
G*W*D*H	NS	NS	NS	NS	F=1.36; p=0.0346	NS
	SGROOM	D	Act_struct	ACT_COW	AGON	EXPL_pass
Group (G)	NS	F=31.61; p=0.023	NS	NS	F=12.02; p=0.0122	NS
Cow	F=8.63; p=0.0001	F=4.40; p=0.0001	F=3.31; p=0.0008	F=2.68; p=0.003	NS	F=2.8; p=0.002
Week (W)	F=3.09; p=0.0152	NS	NS	F=3.56; p=0.0067	F=3.55; p=0.0069	NS
Day (D)	NS	F=6.1; p=0.0023	NS	NS	NS	NS
Hour (H)	F=13.97; p=0.0001	F=14.68; p=0.0001	F=9.84; p=0.0001	F=5.68; p=0.0001	NS	F=145.49; p=0.0001
G*W	NS	NS	NS	NS	F=4.44; p=0.0014	NS
G*D	NS	NS	NS	NS	NS	NS
W*D	F=7.38; p=0.0001	NS	NS	NS	F=2.15; p=0.0287	F=9.66; p=0.0001
G*W*D	F=2.83; p=0.0041	NS	F=3.02; p=0.0023	NS	NS	NS
G*H	NS	F=4.89; p=0.0001	F=3.02; p=0.0023	NS	NS	F=5.58; p=0.0001
W*H	NS	NS	F=1.71; p=0.0082	NS	NS	F=3.83; p=0.0001
G*W*H	NS	NS	F=1.57; p=0.0226	NS	NS	NS
D*H	NS	F=1.66; p=0.0487	F=1.85; p=0.0208	NS	NS	F=2.50; p=0.0009
W*D*H	F=1.77; p=0.0002	F=1.68; p=0.0008	F=1.89; p=0.0001	NS	NS	F=5.01; p=0.0001
G*D*H	NS	NS	F=1.68; p=0.0438	NS	NS	NS
G*W*D*H	NS	NS	F=2.05; p=0.0001	NS	NS	F=1.58; p=0.0029
	VOC	Elimin	TP	T	TOT_l	TOT_s
Group (G)	NS	NS	NS	F=12.07; p=0.0047	NS	NS
Cow	F=31.53; p=0.0001	NS	F=16.45; p=0.0001	NS	F=10.61; p=0.0001	F=10.7; p=0.0001
Week (W)	NS	NS	F=3.18; p=0.013	NS	F=6.37; p=0.0001	F=6.45; p=0.0001
Day (D)	NS	NS	NS	NS	NS	NS
Hour (H)	NS	F=2.42; p=0.0135	F=3.81; p=0.0002	NS	F=47.74; p=0.0001	F=48.73; p=0.0001
G*W	NS	NS	F=2.66; p=0.0311	F=2.88; p=0.0218	NS	NS
G*D	NS	NS	NS	NS	NS	NS
W*D	NS	F=2.05; p=0.0375	NS	NS	F=4.47; p=0.0001	F=4.41; p=0.0001
G*W*D	NS	NS	NS	NS	NS	NS
G*H	NS	NS	F=2.13; p=0.0301	NS	F=3.96; p=0.0001	F=4.12; p=0.0001
W*H	NS	NS	NS	NS	F=1.58; p=0.0221	F=1.54; p=0.0273
G*W*H	NS	F=1.85; p=0.0035	NS	NS	NS	NS
D*H	NS	NS	NS	NS	F=2.14; p=0.0014	F=2.3; p=0.0025
W*D*H	NS	NS	NS	NS	F=1.95; p=0.0001	F=1.95; p=0.0001
G*D*H	NS	F=1.81; p=0.0249	NS	NS	F=1.69; p=0.0429	F=1.69; p=0.0433
G*W*D*H	NS	NS	NS	NS	NS	NS

different combination of hour, day, week and group. Thus, on the behavioural categories which were different depending on $GWDH_{iklm}$ a further analysis was performed. For this further analysis, only data pertaining the three days in which water was withdrawn (Tuesday in the OC, and Tuesday and Thursday in the RC) and their control (Tuesday in the CT-OC, and Tuesday and Thursday in the CT_RC) were used, in order to control for environmental confounding, as the two groups acted as control of one another during those days. Furthermore, three hour-classes were chosen:

1) during late water withdrawal (the mean of 07:30 and 09:00 (half)hour periods),
2) immediately after water withdrawal (the mean of 09:30 and 10:00 (half)hour periods) and
3) away from water withdrawal (the mean of 13:00 and 13:30 (half)hour periods; i.e., from 13:00 onward).

The three above mentioned hour-classes were chosen because they were deemed to be the most representative of water unavailability (as the condition had been going on for at least 1.5 hours), relief of water restriction and normal management.
For each cow, a mean for the three days in which water was withdrawn and one for the three days in which it acted as control was calculated for each hour-class. Means during water restriction days vs means during days of control condition were then compared using a Wilcoxon test for matched data for each hour-class. The same procedure was applied to the behaviour "Drinking" irrespectively of the GML result in order to check whether there was a rebound effect when water withdrawal was discontinued.

Automatically recorded general activity - The data concerning the automatically recorded general activity pertaining the Tuesday and Thursday of the experimental weeks were analysed using repeated measure ANOVA, where the repeated variable within subject (cow) was hour (the 24 hour of the day), and the independent variables were Cow, Group, Week, Day (two levels, Tuesday and Thursday). The effects of the following interactions were also tested: G*W, G*D, W*D, G*W*D.

Milk production - The effects of the stress on milk production parameters (production, peak flow, average flow, milking duration) were tested using ANOVA and considering Cow, Group, Week, Day (three levels, Tuesday, Thursday and Saturday) and Hour (two levels: morning milking and afternoon milking). The effects of the following interactions were also tested: G*W, G*D, W*D, G*W*D.

Cortisol concentrations - Cortisol plasma concentrations were analysed using ANOVA, and considering Cow, Group, Week, Day (two levels, Tuesday and Thursday). The effects of the following interactions were also tested: G*D, W*D, G*W*D.

RESULTS

Instantaneous scan sampling behavioural data

The results of the analysis performed on the behavioural instantaneous scan sampling data are detailed in Table 4. The behaviour "other" is omitted, as it was never recorded.
The interaction Group x Week x Day x Hour ($GWDH_{iklm}$), which could reflect the application of the experimental water withdrawal, influenced the prevalence of "Activities towards structures", "Movement" and "Passive exploration".
For these behavioural categories, the results of the Wilcoxon test for the different hour-classes are detailed in Table 5. A rebound drinking activity was also found, as cows undergoing the change in management drank more than their control counterpart did (Z=2.9; p=0.003) as soon as water withdrawal was lifted, whereas no difference was found from 13:00h onward (Z=0.5; p=0.66).

Automatically recorded general activity

The automatically recorded general activity was not affected by water unavailability, but presented marked circadian variations with three peaks (the first between 08:00 and 12:00; the second between 14:00 and 16:00, and the third between 18:00 and 20:00). Moreover, cows in Group 1 showed an overall significantly higher level of activity during the whole experiment (p=0.002) (Fig. 1).

Milk production

Milk production parameters were not affected by treatment as represented by the interaction G*W*D. Time spent milking differed by week (F=16.06; p<0.0001) and by the interaction G*W (F=3.02; p=0.02). Average milk flow differed between groups (F=110.3; p<0.0001) and week (F=5.8; p<0.0001), whereas peak milk flow differed only between groups (F=98.3; p<0.0001). Daily milk production was not significantly affected by any of the studied variables.

Cortisol concentrations

Cortisol concentrations did not show significant differences for the G*W*D interaction, which reflected the application of water withdrawal in the analysis. Also, cortisol concentrations were always within physiological ranges (2-4 ng/ml). Cortisol concentrations were influenced by Group (F=27.7;

Table 5 - Analysis by hour-class (only significant results detailed).

	Hour-class 1: during late water withdrawal		Hour-class 2: immediately after withdrawal lifted		Hour-class 3: from 13:00 onward	
	Z (p)	Direction	Z (p)	Direction	Z (p)	Direction
Act_struc	Z=2.2 (p=0.025)	WW>CONT	NS	–	NS	–
Mov	Z=2.5 (p=0.015)	WW>CONT	Z=2.0 (p=0.04)	WW>CONT	NS	–
EXPL_pass	NS	–	NS	–	NS	–

WW=during water withdrawal days; CONT=during control days.

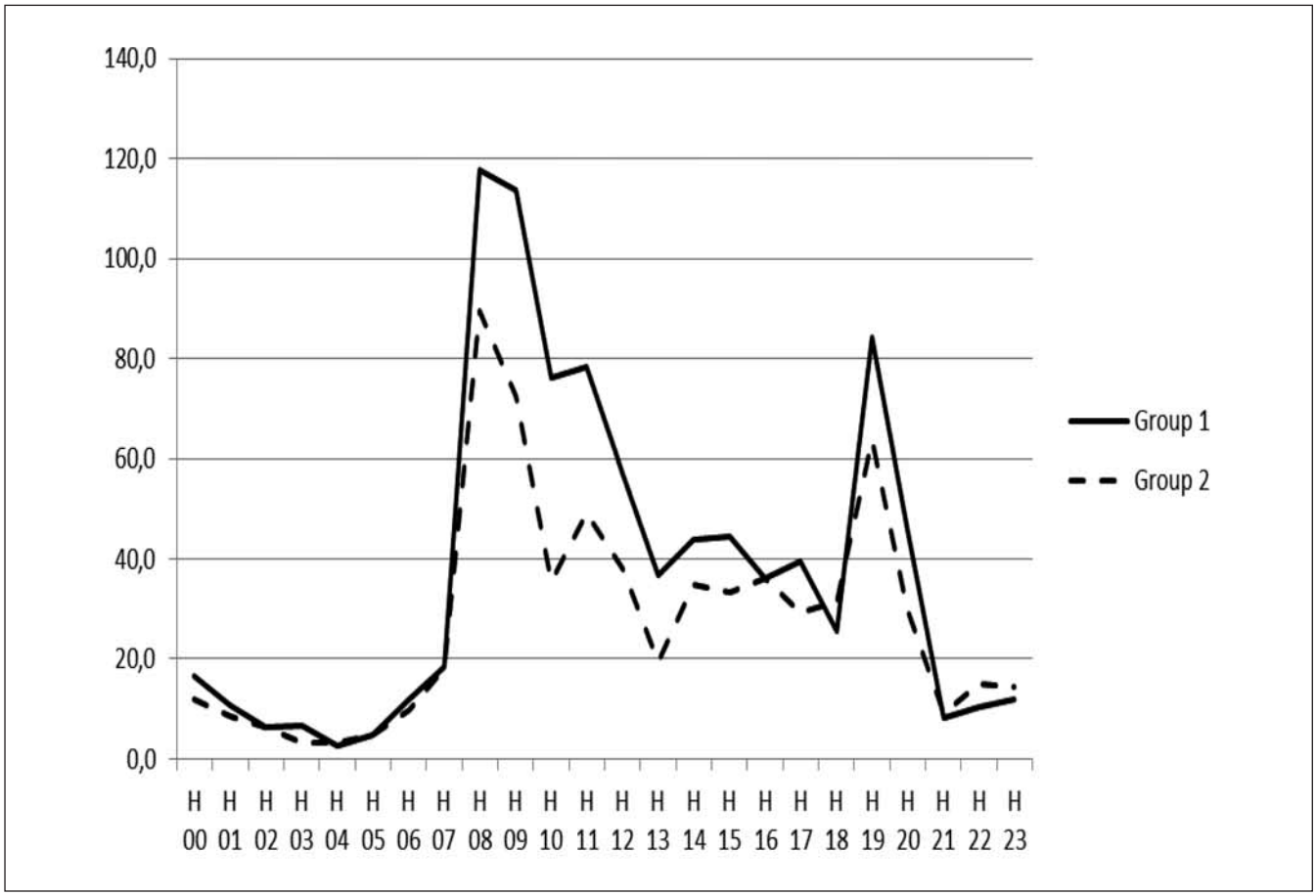

Figure 1 - Circadian pattern of the automatically recorded general activity (movements/hour).

$p<0.001$), with group 2 having higher concentrations than group 1. There was also a significant effect of the week ($F=6.2$; $p<0.001$).

DISCUSSION

During water withdrawal, the cows increased their activities towards structures, which included the drinking equipment. Also the significant effect of the procedure on "movement" could be representative of locomotion to the structures (drinking apparatus in particular) in order to interact with them. In a similar situation[9], i.e., eight-week-old calves not receiving food at the hour they were used to, the increase of activity towards the resource delivering structure (i.e., "head through barriers" behaviour) was interpreted as anticipatory behaviour, due to the fact that the animals had learned to predict the availability of the resource (i.e., milk replacer delivery), and that their expectations were not fulfilled. The increase in activities towards structures during the hours of water withdrawal, found in the present study, could, therefore, have had a similar meaning: cows were used to be able to drink whenever they operated the drinking bowl/equipment, and were experiencing the situation in which the usual action was not resulting in the possibility to drink. An unfulfilled anticipation would, in most cases, lead to some levels of frustration in animals[9], although it is difficult to ascertain whether such a level of frustration could cause significant stress to the cows in the present study. In this regard, a comparison with the result of a similar study performed at the same experimental farm and testing the effects of food delay[10] in dairy cows could be of use, slight differences in experimental design notwithstanding. Cows experiencing[10] a two hour delay in TMR delivery, increased their activities towards the feeding rack, in a way reminiscent of what found in the present study towards structure. However, in the study about food delivery delay, cows also showed an increase in agonistic activities when the delay was repeated for three consecutive days. Although mainly due to the behaviour of few more sensitive animals, the increase in agonistic behaviour was suggestive of the fact that at least some of the cows were experiencing a level of stress. In the present study, agonistic behaviour did not appear to vary according to interaction of independent variables representative of water withdrawal (especially G*W*D which would reflect a significant difference on the last day of the RC, similar to what found in the feed delivery delay study).

It is interesting to note that the increase in movement recorded in the direct behavioural observation during and immediately after water withdrawal was not enough to affect automatically recorded general activity. This finding is important, because if it had it may have represented a confounding to take into account for oestrus monitoring.

The lack of effect on feeding behaviour disagrees with literature, in which a decrease in appetite, feeding behaviour and/or food intake is a general finding[e.g. 11;12;13], together with a decrease in milk production[e.g., 12;3;13]. However, in the present experiment water deprivation was less severe and prolonged than in the aforementioned studies (e.g., 8 days of 25 and 50% restriction of drinking water in[12], watered twice or thrice a day in[13]). Moreover, it has been shown that dairy cows' drinking beha-

vior is associated with the warmer hours of day in winter[14]. In the present experiment, water withdrawal took place relatively early in the morning, which was not the warmest period of the day. During early morning, the cows could have been not strongly motivated to drink, and thus either they were not attempting to reach the drinking apparatus at all, or, even if they attempted to drink, they could have been perceiving water unavailability only as mildly frustrating.
Indeed, apart from a temporary increase in movement and activities towards the structures, which could be a sign of a mild frustration, the 3.5 hour water unavailability during winter months, investigated in the present study, did not significantly alter directly observed behaviour, automatically recorded general activity, plasma cortisol concentration and milking parameters.

CONCLUSIONS

The applied experimental procedure, i.e., a 3.5 hour water unavailability during winter months, did not appear to cause major behavioural changes suggestive of a stress state in the dairy cows. However, the cows did move around more and interacted more with structures, which is suggestive of a state of unfulfilled expectations. No significant changes were seen in cortisol concentrations and productive parameters.

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Feeding a free choice energetic mineral-vitamin supplement to dry and transition cows: effects on health and early lactation performance

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SUMMARY

Introduction - Diets or total mixed rations (TMRs) formulated for specific groups of animals are not always able to satisfy the nutrient requirements of individuals cows and this limit can became critical in key phases of the productive-reproductive cycle such as the dry and the transition periods, whose proper management is recognized to affect the entire subsequent lactation. Various nutritional strategy have been tested for the conduction of these critical phases and different tools have been developed to help farmers in preventing the metabolic and reproductive diseases typical of the fresh cow.

Aim - The aims of the present work were to evaluate the use of a free choice energetic mineral-vitamin supplement as a tool for the nutritional management of dry and transition cows and to study its effect on cattle health and early lactation performances.

Materials and methods - A randomized complete block design was performed on 20 Italian Holstein cows divided into two balanced groups: Control, fed hay and total mixed ration (TMR) and Treated, offered the same diet and the supplemental product as *ad libitum* licking feed. Group feed intake and individual dry matter digestibility, health, metabolic parameters, and productive and reproductive performances were monitored from the drying off to 100 days in milk (DIM).

Results and discussion - The TMR was a fibrous mix with high level of NDF, low level of NFC and an average level of starch, while hay was a mixed hay characterised by a prevalence of grasses. The Treated group consumed from 0.34±0.12 to 0.74±0.50 kg/d of supplement and in comparison with the Control group showed an higher hay intake during the colostral phase (5.98±0.44 vs 4.39±0.57 kg, P = 0.004) and an higher TMR intake during the *post-partum* period (16.16±1.43 vs 14.49±1.35 kg, P = 0.001). No differences were observed in dry matter digestibility at the intervals considered. Because of the higher quantity of energy and nutrient availability, treated animals showed a trend for a lower incidence of ovarian cysts in the fresh period, produced a significantly higher quantity of colostrum (15.4±2.1 vs 5.8±2.8 kg; P = 0.010) and showed a tendentially higher immunoglobulin output (1403±222 vs 706±190 g; P = 0.069) such as tendentially higher cumulative milk production at 20 DIM (632±104 vs 517±134 kg, P = 0.058). Calving to first cycle interval was significantly reduced by treatment (19.8±2.0 vs 41.7±5.2 days; P = 0.001).

Conclusions - The use of free choice energetic mineral-vitamin supplements administered as *ad libitum* dietary component during dry and transition periods seems to be a viable tool for the nutritional management of the dry and transition cows. Under sub optimal nutritional field conditions, this practice appeared to positively affect dry matter intake, colostral phase performance and early lactation health status but also reproductive function resumption and milk production.

KEY WORDS

Ruminant, dairy cows, supplementation, free choice, transition period.

INTRODUCTION

In commercial dairy farms, diets or total mixed rations (TMRs) formulated for specific periods of the productive-reproductive cycle of dairy cattle are not always able to satisfy the nutrient requirements of individual cows. Moreover, factors like sorting and management issues, such as overcrowding and wrong total mixed ration (TMR) preparation, can affect the possibility of dairy cows to satisfy their nutritional needs. During the dry and transition period this limitation can be really dangerous and compromise the health status and the subsequent dairy cattle performances. The dry period management has been demonstrated to deeply affect the following lactation, and the transition period has been recognized as a critical time in relationship to the rapid metabolic changes that undergo in dairy cattle and to the increased probability for cattle to develop periparturient health problems, as recently reviewed by Roche et al.[1]. One of the main issues during the weeks preceding parturition is the decline of dry matter intake (DMI), strongly affected by diet composition and feeding management[2], which reduces the possibility for cattle to satisfy the requirements for fetal

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development and growth and lactogenesis[3]. Immune dysfunction and oxidative stress are other phenomena characterizing the transition period, related to stress of physical, hormonal and metabolic origin[1]. Mitigation strategies are represented by the dietary supplementation with antioxidant, trace elements and poly-unsaturated fatty acids (PUFA)[4]. Also the mineral equilibrium needs to be considered during the transition period. Calcium, magnesium, phosphorus and potassium administration and blood levels have been observed to play a role in determining milk fever in the post-partum period, and their correct administration can prevent different post-partum diseases[5]. Attempt to sustain DMI and satisfy requirements for both macro and micronutrient in transition cows have been conducted in various ways, both through dietary supplementation in TMR or feedstuff, injection or specific drench in the post-partum, with variable results[6]. However, the quantity of nutrients administered or available to the cattle as well as the bunk management is heavily influenced by farm operators through diet formulation, preparation and distribution pattern or by direct administration and does not necessarily satisfy the animal requirements and behavioral needs. To overcome these limitations, the offer of free choice licking blocks to the animals has been proposed. However, the use of free choice feed additives in dairy cattle nutrition has been tested in the last decades only by few researchers. Muller[7] demonstrated a variable consumption of free choice minerals in lactating dairy cattle. The same author found little evidence that dairy cows offered free choice minerals and vitamins were able to regulate their intake on the base of a specific appetite or need. Ishler[8] sustained that free choice feeding could be applied on pasture but has many limitations, and this practice generates only little or no benefit for dairy producers. More recently, in a study on mineral lick microelement supplementation in dairy cows, Krys et al.[9] showed a variable consumption of the free choice supplement by the animals and no significant increase of the concentrations of the monitored microelements in the cows blood, colostrum and milk. However, these studies were conducted essentially on mineral additives, which not always are fully palatable to the animals and can lead, as demonstrated, to irregular consumption behavior. The inclusion of molasses in the blocks formula has been suggested to increase their consumption by Yuzhi et al.[10] in a study on the use of multinutrient lick blocks for dairy animals, containing molasses, urea, corn meal and minerals in various forms. Today, various typology of licking blocks, claimed for both cattle on pasture or in intensive livestock, are available on the market. They are in general formulated to avoid excessive intake of nutrients at specific ranges of daily consumption.

We hypothesize that feeding a free choice energetic mineral-vitamin supplement containing nutrients that have been demonstrated to be helpful to dairy cattle during dry and transition period could be a functional tool to complete the diet of the animals and to improve health and productivity during early lactation. The aim of the present work was to evaluate the use of a supplemental product supplying both energy - molasses and fat -, vitamins and minerals in the form of low moisture blocks as free choice *ad libitum* licking feed during dry and transition period and to study the effects of this practice on dairy cattle health and early lactation performances.

MATERIAL AND METHODS

The present study complied with Italian laws on animal experimentation and ethics (DL 04/03/2014 n. 26).

Products tested

The products tested were free choice supplements (FCSs) formulated for the dry (FCS1) and for the transition periods (FCS2) as low moisture blocks comprised of a crystallized blend of molasses, proteins, fats, vitamins and trace minerals and administered *ad libitum* as solid licking feeds, whose composition, as declared by the producer (Crystalyx® Products GmbH, Münster, Germany), is reported in Table 3. They were marketed as blocks of parallelepiped shape of the weight of 80.0 kg inserted in plastic containers to be placed in the manger. Because of their crystallized structure, they were declared to be stable to the weather events by the producer. For FCS1 was suggested a maximum intake of 1.5% DM while FCS2 advised maximum intake was set at 2% of dietary DM.

Experimental design and diets

The trial was performed on 20 Italian Holstein cows from a commercial herd of 180 animals located in the north of Italy (45°11'00.9"N 10°26'50.1"E) and conducted as a randomized complete block design. The experiment was carried out from middle July to December, using cattle due give birth around the end of September. According to parity, previous lactation length and milk yield, body condition score (BCS) and expected calving date, cows were allocated to one of two homogeneous groups (Control and Treated) of 10 animals each (2.7 vs. 2.5 lactations, 26.4 vs. 26.1 kg previous lactation average daily milk production, 43.0 vs. 43.5 days dry, 392.8 vs. 420.4 days interpartum, 3.18 vs. 3.05 BCS). The animals of both Control and Treated groups were observed for a period of about 150 days, from the drying off (around 45 days from the expected calving date) to the pregnancy diagnosis (around +100 days in milk - DIM). During the far off period, the Control cows were fed mixed hay *ad libitum* and 5 kg of fresh cows TMR (Table 2), including a standard mineral vitamin premix. From 10 days to the expected calving to parturition cows received 10 kg of fresh cow TMR and mixed hay *ad libitum* while from parturition to 3 DIM cows were offered 20 kg of fresh cow TMR and mixed hay *ad libitum*; in the following period only TMR was fed. The animals of the Treated group received the same diet of the Control animals and were offered FCS1 from -45 to -10 days from the expected calving date, and FCS2 from -10 to +20 DIM. The animals of the Control and Treated groups were housed in two separated paddocks from drying off to 10 days from the expected calving date, then in two separated transition pens from -10 to +20 DIM. Each transition pen was composed of three separated areas: the close up area, where cattle were housed from the end of the far off to parturition, the calving area housing cows during the first 3 days of lactation (colostral phase) and the post-partum area where they were kept from 4 to 20 DIM (*post-partum* period). After this period cows were introduced in the fresh cows group, housed in large free stall, for the lactation period. At the proper intervals relative to the expected calving date (during pre-partum) or to the calving date (after parturition) cows were moved from an area to the other, before feeding, in the morning.

Samples collection and measurements

Feed sampling and analysis - The TMR and mixed hay samples were collected twice a month and analyzed for crude protein (CP), ether extract (EE), dry matter (DM), ash, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), lignin (ADL), and starch; Non Fibrous Carbohydrate (NFC) were calculated by difference to 100%. The main macrominerals were determined by spectrophotometer analysis for the Dietary Cation-Anion Difference (DCAD) estimation, based on the equation $(Na^{+}+K^{+})-(Cl^{-}+S^{2-})$, as reported in the NRC[11]. Diet and forage in vitro NDF Digestibility (NDF-D) was measured at 24 hours of fermentation in a batch system using rumen fluid collected from a dry cow fed two kg of concentrate per day and given *ad libitum* access to grass/alfalfa mixed hay. The indigestible NDF (uNDF) was obtained by fermentation of 240 hours by the same system[12] and employed for the estimation of *in vivo* apparent Dry Matter Digestibility (DMD).

Feed intake and digestibility - Hay intake was the only variable in feed intake during far off, close up and colostral phase while TMR intake was the only variable in feed intake during and after post-partum phase. In the Treated group FCS1 and FCS2 intake were additional variables in calculating feed intake. In the different areas, group feed intake was measured daily on the base of the quantity of feed administered, previously measured, and refusals in the manger (for hay and TMR) or residuals in the container (for FCSs). This difference was weighed for the number of animal in each specific area on a daily base, obtaining a weighted average feed (hay, TMR and FCSs) consumption per day and per area for each group. Energy intake was evaluated for the average individual cow of each group using the CNCPS vers. 6.1 on the base of diet formula and weighted average feed intake per day and per area. Estimation of *in vivo* apparent DMD was performed using uNDF as an internal marker in diet and feces, assuming that an equal amount of uNDF is introduced in the digestive tract with the diet and excreted in feces by cattle. The in vivo apparent DMD was then calculated as 1-(uNDF in diet / uNDF in feces)*100, where uNDF was expressed as % of the DM.

Health status, metabolite determination and fecal scoring - General and reproductive tract health status were monitored daily and during the first and second routinary gynecological visits conducted at 14 to 21 and 28 to 35 days after parturition respectively, including a ultrasonographic evaluation of the reproductive tract performed by a specialized veterinary practitioner. The prevalence of metabolic diseases and pathologies was monitored as key indicator of diet adequacy. Blood samples were collected from the coccygeal vein on the animals of both groups approximately 4 hours after feeding, at 3, 20, 60 and 100 DIM to evaluate the relative metabolic profile. Clinical chemistry examinations included bilirubin (Bil), GOT, GGT, CK, total protein (PT), albumin (Alb), urea, glucose (Glu), cholesterol (Chol), triacylglycerol (Trig) and minerals (Ca, Mg and P). NEFA and BHBA were also determined with the aim to monitor energy metabolism of the cows.

Fecal scoring was performed to evaluate the digestive process on the animals of both groups at drying off such as at +3, +20, +60 DIM and +100 DIM on a scale from 1 (loose) to 5 (hard). Fecal samples were collected at the same intervals, frozen at -20 °C and subsequently used for the estimation of *in vivo* apparent DMD.

Body condition scoring and reproductive performances - Body conditions were scored on the cows of both groups at drying off such as at 3, 20, 60 and 100 DIM. The BCS were assessed by the same observer according to a scale of 1 (thin) to 5 (fat). Calving to first cycle (based on routinary gynecological visits and on activometer data), calving to first detectable heat (based on cattle behavior - checked three times per day - and subsequent gynecological visit) and calving to first service intervals were measured to study the effects of the administration of FCSs on fertility. The voluntary waiting period in the farm was set to 40 days.

Colostrum and milk sampling and analysis, milk yield - Individual samples of colostrum were taken from cows at the first milking time within 2 hours after parturition and individual samples of milk were taken from cows at 20, 60 and 100 DIM. Colostrum evaluation included chemical analysis and immunoglobulin titration. Milk was analyzed for fat, casein, lactose, pH, titratable acidity, urea and somatic cells content at 20 DIM while at 60 and 100 DIM, milk was assayed only for fat and protein. Milk yield was measured twice a day on each cow during the milking process. Fat, lactose and protein were determined by infrared analysis using a Milko-Scan FT6000 (Foss Electric, Denmark); casein content was evaluated using the same instrument by Fourier Transformed Infrared (IR) Spectroscopy. pH was determined with a potentiometer, while titratable acidity was evaluated with 0.25 M-NaOH using the Soxhlet-Henkel method. Somatic cell count was determined with Fossomatic (Foss, Hillerød, Denmark); urea titration was performed by enzyme reaction catalysed by urease, using Bun Analyzer 2 apparatus, by means of P/N 667510 kit Bun reagent. Immunoglobulin titration in colostrums was performed by semi-automated agarose gel electrophoresis system (Hydrasis®, Sebia Electrophoresis, Norcross, GA). Productivity data were electronically collected by the farm management software (Afifarm Herd Management Software®, Afimilk Ltd., Israel) and used for statistical analysis. Based on milk fat and protein content, milk productions at 20, 60 and 100 DIM were corrected for energy content (energy corrected milk - ECM).

Statistical analyses

Statistical analysis was performed using the SPSS for Windows software package (version 21.0; SPSS Inc., Chicago, IL). *Pre-partum* hay intake and *post-partum* TMR intake were compared between groups longitudinally through the ANOVA univariate procedure applied on the weighted average consumption measured daily in the different areas. Colostrum parameters and milk parameters at 20 DIM were compared using the univariate procedure of the general linear model (GLM) applied on data from the single cows. Blood and milk parameters in the following intervals were analyzed using group and interval as fixed factors through the multivariate procedure of the GLM; the single cow was used as experimental unit. *In vivo* apparent DMD and BCS at the different intervals were evaluated between groups using the General procedure of the GLM. The differences between the cu-

mulative prevalence of disease in the two groups were evaluated using the Fisher's exact statistic test.

RESULTS

Diet composition, feed intake and diet digestibility

TMR composition was substantially constant during the trial. At the visual observation during feeding time, all the cows of the Treated group appeared to have access at the FCSs container and to consume the product. The weighted average intake of the free choice supplements was found to be 0.34±0.12, 0.74±0.50, 0.50±0.31 and 0.53±0.26 kg during the far off, close up, colostral phase and post-partum respectively (Table 1).

No significant differences between groups were observed in hay intake during the far off (Control: 8.59±2.00 kg; Treated: 7.75±1.82 kg) and close-up periods (Control: 4.49±0.91 kg; Treated: 5.02±1.17 kg) while Treated animals showed a significantly higher (5.98±0.44 vs. 4.39±0.57; P = 0.004) average mixed hay intake in response to the treatment during the first 3 days of lactation (colostral phase). TMR intake was significantly higher for the Treated group during postpartum period (16.16±1.43 vs 14.49±1.35; P = 0.001). By direct assumption of the products tested, the animals of the Treated group ingested additional doses of nutrients, including minerals and vitamins (Table 3). Based on estimates made with the CNCPS model, the energy intake was on average increased in treated animals during both prepartum (+0.92 MJ/cow/d) and postpartum periods (+10.38 MJ/cow/d) even if during the far off period the daily energy intake of treated animals appeared lower than the intake of control animals (-1.51 MJ/cow/day). The same parameter resulted higher in treated animals during close up (+9.45 MJ/cow/d) such as during colostral phase (+3.00 MJ/cow/d) and *post-partum* phase (+10.00 MJ/cow/d) - Table 1. Treated animals showed similar average diet digestibility to control animals. In particular, *in vivo* apparent DMD was 68.83±0.59 vs 67.66±0.84% at 3 DIM, 68.64±0.45 vs 68.78±0.64% at 20 DIM - when the treatment ended -, 68.47±1.37 vs 64.45±4.64% at 60 DIM and 69.45±0.78 vs 69.61±0.31% for Treated and Control animals respectively at 100 DIM, when the treatment was already finished.

Health status, metabolic profile and fecal score

No calving problems (e.g. incomplete cervical dilation, uterine inertia, dystocia and uterine/vagina tear) were observed in cattle of both Control and Treated group. Clinical evaluations performed at 15 DIM (Table 4) showed a trend for a lower number of healthy animals in the Control group compared to the Treated group (2 vs. 7; P = 0.070). At 30 DIM the number of ovarian cysts was tendentially lower (0 vs. 4; P = 0.087) in the Treated group. The metabolic profiles of both Control and Treated groups are summarized in Table 5 and 6. NEFA and BHBA resulted numerically lower in the treated cows than in control cows at day 3 (0.32±0.05 vs. 0.37±0.08

Table 1 - Free choice supplements (FCS 1 and 2) intake of the Treated group and effect of FCSs availability or not availability on hay and TMR intake (kg/cow/d) of cows during far off and transition periods[1].

Interval	Phase	Feed	Intake (kg/cow/d)		SEM	Significance level[3]
			Control Mean±sd	Treated[2] Mean±sd		
DIM -45 to -10	Far off	Hay TMR FCS1[4] dEn.(MJ/cow/d)[5]	8.59±2.00 2.37 –	7.75±1.82 2.37 0.34±0.12 -1.51	0.418 – –	0.329 – –
DIM -10 to 0	Close up	Hay TMR FCS2[6] dEn.(MJ/cow/d)	4.49±0.91 4.75 –	5.02±1.17 4.75 0.74±0.50 +9.45	0.301 – –	0.405 – –
DIM 0 to 3	Colostral phase	Hay TMR FCS2 dEn.(MJ/cow/d)	4.39±0.57 9.50 –	5.98±0.44 9.50 0.50±0.31 +3.00	0.344 – –	0.004 – –
DIM 3 to 20	Post-partum	Hay TMR FCS2 dEn.(MJ/cow/d)	– 14.49±1.35 –	– 16.16±1.43 0.53±0.26 +10.00	– 0.284 –	– 0.001 –

DM = dry matter; TMR = total mixed ration; FCS = free choice supplement; SEM = standard error of the mean.

[1] Cows were assigned to Control and Treated groups in a randomized complete block design; Control group cows were offered basal diet while Treated group cows were offered basal diet and free choice supplement.

[2] Free choice supplement with specific composition for dry and transition cows were offered during the Far off (from -60 DIM to -10 DIM) and Transition (from -10 to +20 DIM) periods respectively.

[3] Statistical analysis was performed only on Hay consumption data, since it was the only variable between groups during Far off, Close up and Colostral phase, only on TMR consumption during Post-partum.

[4] Components: Molasses, Magnesium oxide, Monocalcium phosphate, Palm oil, Sodium chloride, manganese (III) oxide, zinc oxide, copper sulfate, potassium iodide, cobalt carbonate monohydrate, sodium selenite, organic selenium from Saccharomyces cerevisiae, E672, E671, tocopherol acetate.

[5] Differential in Energy intake (dEn): difference between Treated group energy intake and Control group energy intake.

[6] Components: Molasses, Sodium propionate, Magnesium oxide, Monocalcium phosphate, Palm oil, Calcium carbonate, manganese (III) oxide, copper sulfate, zinc oxide, potassium iodide, sodium selenite, cobalt carbonate monohydrate, Saccharomyces cerevisiae NCYC SC 47 (E1702), E672, Cobalamin, Niacin, E671, tocopherol acetate.

Table 2 - Chemical composition of TMR and hay fed to cattle during the far off and the transition periods.

Nutrient	TMR[1]	HAY
DM, % as fed	47.5	84.6
CP, %	15.8	10.2
NEl, MJ/kg	5.98	4.77
ADL, %	3.7	4.7
ADF, %	19.9	30.3
NDF, %	39.9	55.2
NDFD 24 hours (% of NDF)	58.3	40.4
NFC, %	33.1	23.3
Starch, %	24.0	-
Ether extract, %	2.9	1.9
Ash, %	8.3	9.4
Ca, %	1.11	0.58
P, %	0.45	0.28
Mg, %	0.25	0.21
K, %	1.39	2.25
Na, %	0.17	0.13
Cl, %	0.46	0.56
S, %	0.20	0.17
DCAD, mEq/kg of DM	17.3	36.8

[1] Ingredients (% of diet DM): 24.1 corn silage, 12.1 wheat haylage, 7.6 alfalfa hay, 4.2 alfalfa haylage, 3.8 grass hay, 2.9 grass haylage, 22.1 corn meal, 1.9 beet pulp, 1.5 wheat bran, 15.2 sunflower meal, 2.3 soybean meal, 1.2 cottonseed meal, 1.0 mineral vitamin premix, 0.1 calcium carbonate. Vitamin mineral premix contained (per kg of DM): 330 g/kg of Ca, 35 g/kg of P, 20 g/kg of Mg, 0,9 g/kg of K, 70 g/kg of Na, 81 g/kg of Cl, 3 g/kg of S, 1150 mg of vitamin E, 900 IU of vitamin A, 230000 IU of vitamin D, 370 mg of Cu, 12 mg of Se and 3540 ppm of Zn.

mmol/l and 0.67±0.05 vs. 0.77±0.13 mmol/l respectively) while they became numerically higher in the treated cows at day 20 (0.24±0.05 vs. 0.19±0.03 mmol/l and 0.64±0.04 vs 0.54±0.06 mmol/l), at the end of the treatment (Figure 1). In the following intervals, NEFA levels appeared significantly higher in the treated animals at 60 and 100 DIM (0.11±0.01 vs. 0.09±0.01 mmol/l and 0.13±0.02 vs. 0.08±0.01 mmol/l respectively, P = 0.031). A different evolution of BHBA levels was observed between groups from 60 to 100 DIM, with a marked decrease of plasma BHBA in the treated cattle and no variation in the control group, leading to a significant interaction of Group x DIM (P = 0.017). Starting from similar levels between groups at day 3 and 20 *post-partum*, cholesterol showed a dramatic increase at day 60 of lactation, when the difference between Treated and Control groups became statistically significant (206.7±17.1 vs. 161.1±11.6 mmoli/l; P = 0.009) and a subsequent general decline at 100 days of lactation when cholesterol in the Treated group was still higher (Figure 2). In general, triacylglycerol levels appeared similar or lower in the Control group than in the Treated one; the difference between groups resulted significant only in the interval between 60 and 100 DIM when the variation of this parameter appeared differentiated based on the previous treatment, with a decrease that was significantly more pronounced in treated animals (8.25±0.47 vs. 11.15±0.54 mg/dl at 100 DIM; P = 0.028).

No significant differences were observed between groups for minerals in blood; a significant increase over time was observed in both groups for magnesium between day 3 and day 20 (P = 0.006) while a significant decrease was observed in calcium levels between 60 and 100 DIM (P<0.001) in both groups. Variations over time have been observed also for plasma urea, which decreased between 3 and 20 DIM reaching the maximum levels at 60 DIM.

Starting from similar fecal scores at the drying off (2.89±0.12 vs. 2.68±0.10 for Treated and Control groups respectively), cows of the Treated group were found to have a higher fecal score immediately after parturition, with a score difference of almost 0.5 (2.47±0.08 vs. 2.05±0.09). Similar differences were observed also at 60 (3.19 vs 2.86) and 100 DIM (3.02±0.09 vs 2.81±0.07 for Treated and Control groups respectively).

Body condition and reproductive performances

Body condition score of the animals of both Control and Treated groups appeared similar at the various intervals con-

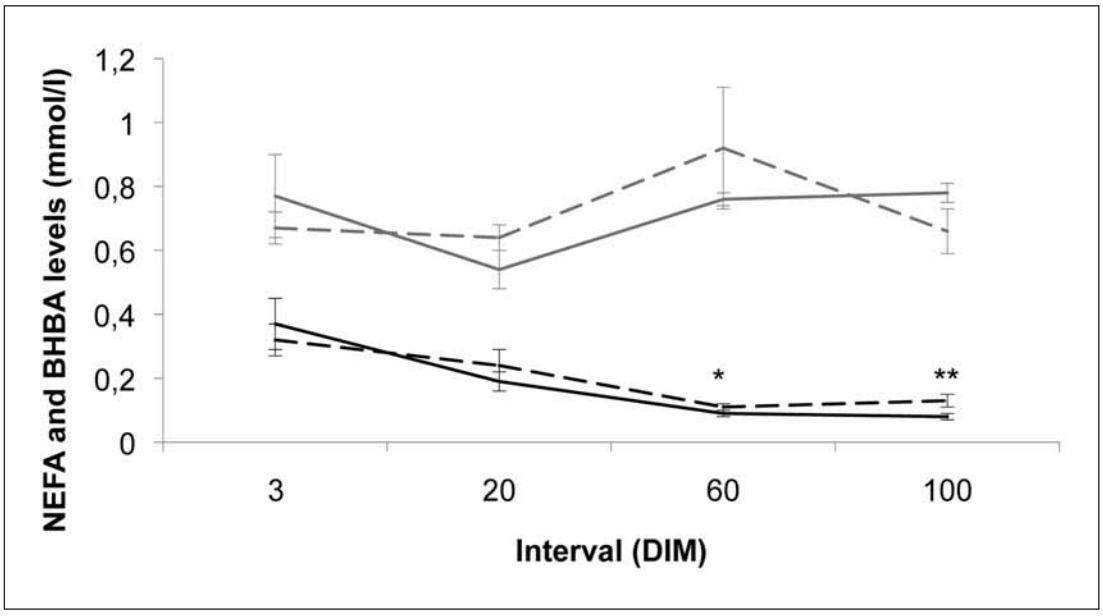

Figure 1
Effects of free choice supplements availability (broken lines, Treated group, n = 10) and not availability (continuous lines, Control group, n = 10) on blood NEFA (black) and BHBA (grey) in cows at 3, 20, 60 and 100 DIM (mmol/l) -
* P<0.1; ** P<0.05.

Table 3 - Declared chemical composition of the free choice supplements offered to the Treated group cows during far off (FCS1) and transition (FCS2) periods and additional doses of nutrient consumed by treated animals.

Item	Chemical composition		Additional doses of nutrients consumed by treated animals[3]	
	FCS1[1]	FCS2[2]	Prepartum	Postpartum
	(%)		g/cow/d	
DM	98	98	429	504
Nutrients	(%DM)		g/cow/d	
CP	4	4	17	20
Fat	2.5	6.0	16	30
Ash	36	28	141	141
Sugars	32	30	134	151
Macrominerals	(g/kg)		g/cow/d	
Ca	1	4	7	20
P	1.3	2.0	7	10
Na	4	3	16	15
Mg	7	1	20	5
Microminerals	(mg/kg)		mg/cow/d	
Mn	2 000	800	6604	4036
Zn	3 000	1 200	9907	6054
Cu	1 000	600	3631	3027
I	200	60	628	302
Co	13	12	54	60
Se + OSe[4]	20	20	86	100
Vitamins	(I.U./kg)		I.U./cow/d	
Vitamin A	150 000	80 000	52822	40360
Vitamin D3	30 000	20 000	11222	10090
Vitamins	(mg/kg)		mg/cow/d	
Vitamin E	2 500	150	686	75,675
Vitamin B12	0	200	33	100,9
Niacin	0	1 000	164	504,5
S. cerevisiae (CFU)[5]	0	150x10^9/kg	0	76x10^9/kg

DM = dry matter; CP = crude protein; OSe = Organic Selenium; CFU = colony forming units.
[1] Components: Molasses, Magnesium oxide, Monocalcium phosphate, Palm oil, Sodium chloride, manganese (III) oxide, zinc oxide, copper sulfate, potassium iodide, cobalt carbonate monohydrate, sodium selenite, organic selenium from Saccharomyces cerevisiae, E672, E671, tocopherol acetate.
[2] Components: Molasses, Sodium propionate, Magnesium oxide, Monocalcium phosphate, Palm oil, Calcium carbonate, manganese (III) oxide, copper sulfate, zinc oxide, potassium iodide, sodium selenite, cobalt carbonate monohydrate, Saccharomyces cerevisiae NCYC SC 47 (E1702), E672, Cobalamin, Niacin, E671, tocopherol acetate.
[3] Values are calculated and reported as group average weighted for the number of cows in the phase each day.
[4] From Saccharomyces cerevisiae.
[5] S. cerevisiae NCYC SC 47 (E1702).

Table 4 - Effects of free choice supplements availability and not availability on the number of cases of diseases in cows at 15 and 30 DIM.

Interval (DIM)	15			30		
Group	Control (n = 10)	Treated (n = 10)	Significance level[1]	Control (n = 10)	Treated (n = 10)	Significance level[1]
M	4	1	NS	1	1	NS
SM	4	1	NS	1	1	NS
OC	1	0	NS	4	0	<0.1
HO	0	1	NS	0	2	NS
L	0	1	NS	0	1	NS
PTP	1	0	NS	–	–	–
HC	2	7	<0.1	5	6	NS

DIM = days in milk (days of lactation); M = metritis; SM = subclinical metritis; OC = ovarian cysts; HO = hypoplastic ovaries; L = lameness; PTP = pre-term parturition; HC = healthy cows. NS = not significant.
[1] The Fisher's exact statistic test was applied.

sidered. The treated cows had slightly lower BCS than the control ones at day 3 after parturition (2.26±0.06 vs. 2.39±0.09) and 20 DIM (1.95±0.09 vs. 2.14±0.10) but numerically higher BCS at 60 DIM, after the peak of lactation (1.80±0.07 vs. 1.70±0.08). Resumption of the reproductive activity appeared to be shortened in the Treated group, as indicated by the calving to first cycle interval, that was significantly reduced by treatment (19.8±2.0 vs. 41.7±5.2 days; P = 0.001). As a consequence, the interval calving to first detectable heat was numerically reduced in the Treated group (44.5±8.7 vs. 52.2±7.8). No differences were observed with regard to calving to first service interval (58.5±6.4 vs 58.5±6.1).

Colostrum secretion and milk production

Animals receiving the free choice supplement secreted a significantly higher quantity of colostrum (15.4±2.1 vs. 5.8±2.8 kg; P = 0.010) and showed a tendentially higher calculated mammary immunoglobulin output at first milking (1403±222 vs. 706±190 g; P = 0.069). No significant differences were found in colostrum composition and properties (Table 7). Dry matter content, Ash, Fat and Crude Protein were numerically higher in the Treated group, which showed lower percentage of lactose. Average milk production at 3 DIM was significantly higher in the Treated group (16.94±1.59 vs. 11.60±1.84 kg/d; P = 0.032). At the end of the treatment (20 DIM), cumulative milk production and average milk production (expressed as ECM) were tendentially higher for the Treated group (632±104 vs. 517±134 kg, P = 0.058; 40.6±4.8 vs. 31.9±3.1 kg/d, P = 0.100). The animals of the Treated group showed tendentially lower level of protein and significantly lower level of casein (2.31±0.06 vs. 2.58±0.05;

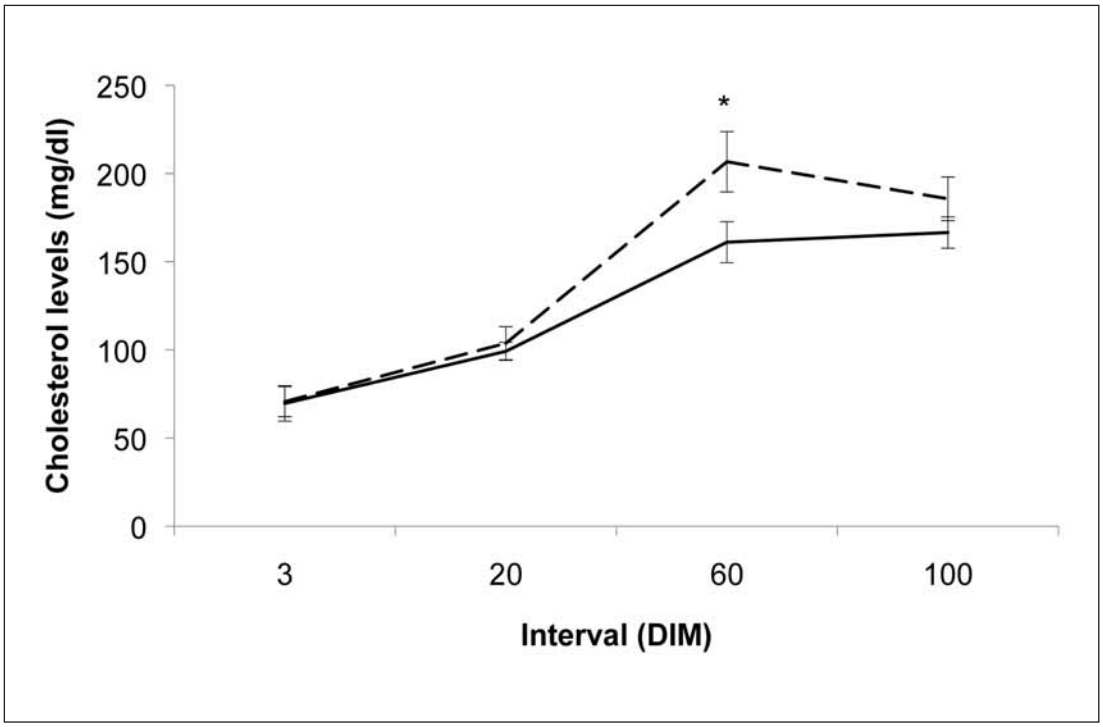

Figure 2
Effects of free choice supplements availability (broken line, Treated group, n = 10) and not availability (continuous line, Control group, n = 10) on cholesterol in cows at 3, 20, 60 and 100 DIM (mg/dl) - * P<0.1.

P = 0.013). After the end of the treatment (20 DIM), a trend for a significant difference of milk production dynamic between groups was observed even if this finding was not confirmed by the data about average ECM production (Table 7).
No significant differences were observed between groups in the following periods (20, 60 and 100 DIM) with regard to milk yield and quality (Table 8).

DISCUSSION

The present work was performed with the aim to evaluate the use of a free choice energetic mineral-vitamin supplement as new tool for the nutritional management of the dry and transition period and to test its effect on early lactation health status and performances in dairy cattle. The study was a controlled field trial conducted offering the product

Table 5 - Effects of free choice supplements availability and not availability on blood parameters in cows and blood parameter values at 3 and 20 DIM. Values are expressed as Least Squares Means ± Standard Error.

	3 DIM		20 DIM		SEM	Significance level		
	Control	Treated	Control	Treated		Group	DIM	Group X DIM
Bil (mg/dl)	0.09±0.02	0.10±0.03	0.07±0.01	0.08±0.01	0.008	0.854	0.639	0.720
GOT (U/l)	28.80±1.76	29.92±1.79	31.2±1.76	32.56±0.79	0.684	0.224	1.000	0.584
GGT (U/l)	11.11±0.84	15.34±1.70	12.08±1.02	12.52±1.38	0.666	0.202	0.422	0.352
PT (g/dl)	6.96±0.14	6.94±0.11	7.17±0.10	6.93±0.36	0.109	0.377	0.877	0.457
Alb (g/dl)	3.27±0.07	3.38±0.07	3.29±0.08	3.38±0.06	0.040	0.206	0.894	0.885
Urea (mg/dl)	25.67±2.10	25.75±2.04	19.83±1.17	23.93±1.82	0.886	0.416	0.029	0.295
Glu (mg/dl)	51.44±1.34	50.65±1.13	49.83±1.77	47.07±1.31	0.784	0.195	0.193	0.704
P (mg/dl)	5.79±0.20	5.14±0.32	5.64±0.28	5.68±0.38	0.150	0.311	0.638	0.356
Ca (mg/dl)	8.72±0.08	8.93±0.09	8.63±0.09	8.74±0.12	0.051	0.239	0.196	0.461
Mg (mg/dl)	2.00±0.05	2.02±0.07	2.22±0.04	2.19±0.04	0.030	0.954	0.006	0.961
Glo (mg/dl)	3.69±0.17	3.56±0.11	3.84±0.11	3.72±0.15	0.061	0.332	0.258	0.754
Chol (mg/dl)	69.64±10.08	70.65±8.53	99.18±5.16	103.75±9.40	3.681	0.500	<0.001	0.703
Trig (mg/dl)	11.08±2.11	11.77±2.61	13.24±1.44	9.6±1.71	0.683	0.409	0.655	0.281
CK (U/l)	60.23±47.21	74.72±26.83	196.47±44.87	80.73±3.64	21.468	0.391	0.380	0.238
NEFA (mmol/l)	0.37±0.08	0.32±0.05	0.19±0.03	0.24±0.05	0.029	0.821	0.047	0.274
BHBA (mmol/l)	0.77±0.13	0.67±0.05	0.54±0.06	0.64±0.04	0.039	0.713	0.288	0.283

SEM = standard error of the mean; DIM = days in milk.

Table 6 - Blood parameters of the fresh cows of the animals of the Treated and Control groups at the peak of lactation (+60 DIM) and after peak (+100 DIM). Values are expressed as Least Squares Means ± Standard Error.

	60 DIM		100 DIM		SEM	Significance level		
	Control	Treated	Control	Treated		Group	DIM	Group X DIM
Bil (mg/dl)	0.07±0.02	0.07±0.01	0.05±0.01	0.07±0.01	0.006	0.661	0.468	0.236
GOT (U/l)	35.46±4.23	32.77±1.83	33.17±2.00	31.73±1.25	1.042	0.501	0.311	0.586
GGT (U/l)	16.89±1.60	21.7±3.22	15.43±1.13	17.29±1.32	0.840	0.054	0.747	0.246
PT (g/dl)	8.04±0.12	7.52±0.08	7.56±0.15	7.55±0.19	0.089	0.392	0.166	0.128
Alb (g/dl)	3.56±0.11	3.63±0.15	3.62±0.08	3.57±0.06	0.042	0.992	0.886	0.557
Urea (mg/dl)	30.47±1.70	35.26±1.15	29.39±1.88	28.65±1.73	0.997	0.711	0.058	0.14
Glu (mg/dl)	52.74±1.16	54.73±2.64	55.77±1.23	56.66±1.28	0.759	0.585	0.102	0.985
P (mg/dl)	6.31±0.23	6.34±0.23	5.96±0.27	5.86±0.29	0.144	0.875	0.276	0.584
Ca (mg/dl)	9.22±0.14	9.36±0.11	8.67±0.09	8.73±0.10	0.065	0.629	0.000	0.726
Mg (mg/dl)	2.03±0.13	2.24±0.05	2.29±0.06	2.15±0.07	0.038	0.816	0.932	0.161
Glo (mg/dl)	4.48±0.21	3.9±0.15	3.93±0.19	3.98±0.22	0.109	0.481	0.232	0.139
Chol (mg/dl)	161.07±11.60	206.68±17.12	166.54±8.87	185.66±12.36	6.727	0.009	0.619	0.162
Trig (mg/dl)	9.04±1.05	9.13±0.58	11.15±0.54	8.25±0.47	0.385	0.028	0.181	0.027
CK (U/l)	249.46±87.80	87.16±8.31	75.06±6.19	105.11±14.80	29.191	0.318	0.259	0.131
NEFA (mmol/l)	0.09±0.01	0.11±0.01	0.08±0.01	0.13±0.02	0.010	0.031	0.896	0.747
BHBA (mmol/l)	0.76±0.02	0.92±0.19	0.78±0.03	0.66±0.07	0.042	0.394	0.366	0.017

SEM = standard error of the mean; DIM = days in milk.

as a part of the diet routinarily employed in the farm. Both FCSs contained a large amount of molasses and their intake expressed as daily weighed average, has been always abundant and quite regular, indicating a good palatability of the products offered. The quantity of FCSs consumed exceeded at all the intervals considered the maximum level indicated by the producer, probably as a consequence of the not adequate plan of nutrition adopted in the farm. As indicated by

Table 7 - Effects of free choice supplements availability and not availability on yield and characteristics of colostrum and milk from cows at 3 and 20 DIM. Values are expressed as Least Squares Means ± Standard Error.

Interval	Item	Group		SEM	Significance level
		Control (n = 10)	Treated (n = 10)		
Parturition	Colostrum yield (kg)	5.8±2.8	15.4±2.1	2.0	0.010
(First milking)	Colostrum composition (%)				
	DM	26.68±1.2	28.97±1.9	1.18	0.460
	Ash	1.01±0.03	1.07±0.05	0.03	0.376
	Fat	3.59±0.40	4.34±0.66	0.45	0.440
	CP	17.40±0.96	19.33±1.55	0.95	0.424
	Lactose	4.69±0.16	4.23±0.11	0.11	0.044
	Colostrum IgG content (g/l)	121.2±13.8	94.8±9.7	9.07	0.344
	Mammary IgG output (g)	706±190	1403±222	173.5	0.069
3 DIM	Average production (kg/d)	11.60±1.84	16.94±1.59	1.255	0.032
	Cumulative production (kg)	34.8±8.0	50.8±6.3	5.271	0.131
20 DIM	Average milk production (kg/d)	29.1±1.8	34.6±2.6	1.8	0.222
	Cumulative production (kg)	517±134	632±104	21.22	0.058
	ECM (kg/d)	31.9±3.1	40.6±4.8	3.0	0.100
	Milk composition (%)				
	Fat	4.79±1.36	5.14±1.05	0.89	0.874
	Lactose	4.74±0.08	4.63±0.09	0.06	0.378
	Protein	3.24±0.08	2.94±0.08	0.06	0.071
	Casein	2.58±0.05	2.31±0.06	0.04	0.013
	Milk UREA (mg/dl)	22.00±1.94	21.42±1.71	1.41	0.859
	Somatic Cells (log_{10} cell/ml)	5.45±4.99	5.45±4.98	4.83	0.830
	pH	6.73±0.02	6.70±0.02	0.01	0.352
	Acidity (°SH)	2.97±0.12	3.09±0.08	0.08	0.478

DIM = days in milk (days of lactation); SEM = standard error of the mean; DM = dry matter; CP = crude protein; Ig = immunoglobulin; ECM = energy corrected milk; °SH = degree Soxhlet-Henkel.

Table 8 - Effects of free choice supplements availability and not availability on yield and characteristics of milk from cows at 20, 60 and 100 DIM. Values are expressed as Least Squares Means ± Standard Error.

Item	Group		Interval (DIM)			SEM	Significance level		
	Control (n = 10)	Treated (n = 10)	20	60	100		Group (G)	Interval (I)	G X I
Milk yield									
Milk (kg/d)	31.3±1.2	34.3±0.9	32.5±1.7	34.3±1.6	31.5±1.3	0.984	0.287	0.153	0.097
ECM (kg)	34.0±1.6	35.6±2.0	34.1±2.8	36.7±1.7	33.7±1.8	1.351	0.609	0.344	0.388
Cum. Prod. (kg)	1817±193	2069±216	578^{a}±26	1960^{b}±79	3290^{c}±127	152.9	0.109	<0.001	0.260
Milk composition									
Fat (%)	4.29±0.46	3.84±0.36	4.31±0.84	4.01±0.15	3.89±0.07	0.312	0.447	0.995	0.872
Protein (%)	3.34±0.04	3.20±0.06	3.13±0.06	3.32±0.06	3.35±0.06	0.039	0.255	0.569	0.451

DIM = days in milk (days of lactation).

the energy concentration data, the TMR administered as a part of the diet was a fibrous mix with high level of NDF, low level of NFC and an average level of starch. Fiber digestibility at 24 hours of fermentation was higher than the maximum value found by Righi et al.[13] for lactating cattle TMR, reducing the potential depressive effect of NDF level on dry matter intake. However, the high level of NDF (and the subsequent low level of NFC) led to a reduced energy concentration of the diet. Ca, P, K and other mineral levels of the TMR appeared generally adequate for lactating cows. Hay composition was typical of a mixed hay characterised by a prevalence of grasses, as indicated by the low level of protein and the high content of NDF. During *pre-partum* period hay represented the main constituent of the diet and TMR was about 1/5 and about 1/2 of the DMI in far off and close up periods, respectively. This increased the NDF level of the daily diet reducing starch proportion and probably avoided the decline in DMI around parturition, according Janovick and Drackley[14]. Hay and TMR intake of the treated animals were significantly higher during the colostral and *post-partum* phases, respectively. Any difference was found with regard to the relative diet digestibility (expressed as DMD) and fecal score between groups. The combined interpretation of data regarding hay/TMR intake - higher in the Treated group - and diet *in vivo* apparent DMD after parturition - similar between groups - suggest an increase in the absolute amount of dry matter digested by treated animals. The increase in hay intake in the Treated group during the colostral phase - that apparently started in the close up - and the increased TMR intake during the following period could therefore be considered as an indicator of increased rumen activity and diet digestion. These effects can be related to the additional doses of components such as molasses received by the animals of this group through the assumption of FCSs. The use of molasses in the diet has in fact been shown to improve fiber digestion, DMI and milk yield in mid lactation cows, probably by a stimulating effect on fiber-digesting bacteria in the rumen[15]. Based on approximate estimations made using the CNCPS model (setting the animal average weight at 680 kg), during the far off period, Metabolizable Energy (ME) requirements were satisfied for about 80-90%, while in the same period Metabolizable Protein (MP) amounted to about 110-120% of the requirements in both Control and Treated groups. These levels were in general slightly reduced, during the close up period since the ME administered to the animals of the two groups amounted to about 70-80% of the requirements. In the following periods, ME requirements were met for about 80-90% and 60-70% by the diets of both groups. It appears that animals in the present trial were generally fed under the requirements; this was a consequence of the nutrition plan adopted in the farm and was not intentional. The diet adopted in the farm was not completely adequate to meet cattle requirements and this could justify the high prevalence of diseases generally observed in both groups. Furthermore, the dietary deficiencies could have to some extent increased the intake and sharpen the effects of the products tested. Ca and P requirements were met by diets of both groups during far off, close up and colostral phase, while they were met only by treated cows during the *post-partum* even if no significant effect of group was observed for these macro-minerals in blood. Animals of both groups showed the same low BCS at parturition followed by a further BCS loss in the *post-partum* period, but the body condition losses of the treated animals were on average slightly lower (0.55 vs. 0.75) around the peak of lactation (60 DIM). This is consistent to their calculated higher energy intake in the *post-partum* period and, as indicated by clinical data, to their better health status in the same interval.

The first two weeks of lactation represent the primary risk period for subclinical ketosis, defined by a serum concentration of BHBA ≥ 1.4 mmol/L, and blood BHBA ≥ 1.2 mmol/L in the first week after calving increased the odds for metritis by 3.4-fold[2]. A further maximum limit of 0.7 mmol/L of NEFA for the diagnosis of subclinical ketosis was previously introduced. In our study, only one control cow at day 3 showed BHBA values higher than 1.4 mmol/l and NEFA higher than 0.7 mmol/L. Numerical variations of NEFA and BHBA levels in the Treated group are probably the effects of a higher energy supply in the immediate *post-partum* (associated with the higher energy intake in the transition period) and of a subsequent higher productivity and energy output that led to a more negative energy balance around the peak of lactation, after the end of the treatment. Starting from 60 DIM, treated animals showed higher levels of cholesterol in plasma indicating a more intense/efficient liver fat mobilization but also higher levels of NEFA indicating a more intense body fat reserves mobilization with respect to the control animals. This process was probably still ongoing at 100 DIM, when treated cows showed significantly lower levels of plasma triacylglycerol

and numerically lower BCS. The higher diet intake and the relative different diet composition related to the assumption of the FCSs such as the higher amount of diet digested and fat usage, together with the improved health status led to the increased cumulative milk production observed in the Treated group during the fresh period. Several micronutrients have been shown to concur in the improvement and maintenance of the reproductive apparatus efficiency[16] and treated cows consumed additional doses of Se, Vit. A and Vit. E whose action has been related to uterine, ovarian and udder health by several authors. Supplemental doses of Zn, Cu and Co have been also shown to play a role in the antioxidant system, whose efficiency relates with immune system functionality and activity[17,4]. Similarly to milk production, also the earlier resumption of ovarian activity can be partially explained by the higher energy and nutrient supply observed in the supplemented cows but the main effect was probably related to the better health status of cattle connected with higher levels of vitamins and trace elements received by the treated animals as previously reported. The low number of animals tested however, reduces the statistical values of the results on health status, so the observations made should be considered as indication of a potential effect of the free choice administration of the product or as a trend for an improved health status.

Despite the potential impact of dry cow nutrition on fresh cattle metabolism[3], in the present study, no treatment effect was found on colostrum quality. A slight, not significant dilution effect was observed on IgG content, that was however higher (almost double) of the minimum level to ensure passive immunity transfer to the calf (50 g/l). A significant effect was observed on colostrum yield at first milking that was higher in treated cattle, leading to a trend for a higher IgG secretion immediately after parturition. From a biological perspective this shows the potential of the treatment in stimulating mammary secretion with positive effects on calf nutrition and passive immunization and on milk production in the fresh cows period. Despite a lower relative level of casein, ECM and cumulative milk production tended to be higher at 20 DIM - end of the treatment - as effect of the increased nutrients availability, as previously reported. An elongation of the treatment over the 20 DIM should probably be addressed with the aim to support the higher production obtained improving energy balance also during the peak of lactation.

CONCLUSIONS

The use of free choice energetic mineral-vitamin supplements for dry and transition cows administered as *ad libitum* dietary component can potentially increase DMI and nutrients availability in dry and transition period, improving early lactation cows performances. In particular, positive effects were observed on mammary immunoglobulin output at first milking. Some evidences were found for a positive effect on milk production, health status and reproductive function resumption. Further studies should be addressed at evaluating individual FCSs and diet intake on a higher number of animals for a more accurate evaluation of the product consumption patterns and the relative effect on DMI and for a better understanding of the effect of this feeding practice on cattle health status and performance. Evaluations should also be extended at the study of the indirect effects on calves born from treated cows early growth and health status.

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VIRBAC

ZAC

In vitro study of bovine uterine contractility at various stages of pregnancy

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SUMMARY

Introduction - Throughout pregnancy, the uterus does not remain quiescent, but displays contractile activity in several species. This is made possible by the presence of interstitial Cajal cells in the myometrium and by the ability of myometrium to contract/relax even under the action of hormonal or neuronal stimuli.

Aim - This *in vitro* study investigates spontaneous uterine contractility in cows during different months of pregnancy (from the first at 30 days to the last at 270 days).

Materials and methods - Basal contractility was evaluated in an isolated organ bath and the functionality of strips throughout the experiment was evaluated by a dose of carbachol (10^{-5} M), which always had to be repeatable (≤ difference of 20%) with the previous administration of the same substance. Amplitude, frequency and area under the curve (AUC) of contractions for each strip were determined by analysing the sequence corresponding to the last 30 min of contraction, from the recording section.

Results and discussion - This *in vitro* study demonstrates a variations in contractile capacity, that was highly dependent on the month of bovine pregnancy. Contractility proved extremely limited in early pregnancy (from 0-30 to 90-120 days) and even more so between 120-150 and 180-210 days, reaching maximum force (amplitude) of contraction between 150-180 days. After this period, it decreases from days 180- to 210 and then rises again from days 240- to 270. The contractility detected in the latter two periods is very unusual: between 210-240 days, it is made up of alternating waves with low and high strength, while the period from 240-270 days is characterized by contractures (high-amplitude, low-frequency and long-lasting). The data suggest that the bovine pregnant uterus does not remain quiescent, but displays contractile activity, and raise the hypothesis that steroid hormones may play a role in the modulation of uterine contractions during pregnancy.

Conclusions - Our results provide a new vision of embryonic and fetal mortality, probably attributable to alterations in uterine contractility and cytokine production.

KEY WORDS

Age of pregnancy; uterus contractility; cattle; progesterone; estrogens.

INTRODUCTION

The uterus is the organ of pregnancy, a time when it undergoes profound, largely reversible changes to facilitate foetal growth[1]. Hence, during pregnancy, the control exercised by progesterone (P_4), together with metabolic and neural changes, keep it in a relative dormant state[2].

It has been shown that despite P_4 secretion throughout pregnancy, the uterus does not remain quiescent, but displays contractile activity in women, ruminants, monkeys, guinea-pigs, rats and rabbits[3].

In pregnant cows uterine contractility has been proven *in vivo*[4,5]. Kündig et al.[5] described the contractions produced by the bovine uterus around parturition through pressure microsensors and electrodes implanted in the pregnant horn. They stated that approximately 3 weeks before calving, the frequency was 0.3 to 0.5 contractions/h, lasting 3 to 30 min, and their amplitude ranged from 60 to 80 mmHg in the caudal part of the uterus, and from 20 to 40 mmHg in its cranial part[5]. In late-pregnant cows, Taverne et al.[4] confirmed these findings with similar instruments. They used the term "contracture" to describe low-frequency (13.6 ± 0.9 contractures/d), long-lasting (12.1 ± 0.3 min) bursts of uterine activity during the last weeks of pregnancy, and to differentiate them from the contractions distinctive of labour[4].

The onset of these contractions, in several species, is made possible by the presence of interstitial Cajal cells (ICC) in the myometrium[6]. These cells are closely associated with axons, smooth muscle cells, immune cells in capillaries and connective tissue, giving the myometrium the inherent capacity to generate spontaneous contractions of myogenic origin[6]. Indeed, their position would give ICCs an important modulatory role in neurotransmission and immune regulation[6].

Moreover, the myometrium is able to contract/relax even under the action of hormonal or neuronal stimuli that may exert an important contractility-modulating action. In this regard, several *in vitro* studies highlight the importance of hormones and neurotransmitters on uterine contractions, in pregnant and non-pregnant cows[7,8,9]. However, those Authors evaluated the effects of these substances in only a few phases of bovine pregnancy (early- or late-

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pregnancy), failing to screen contractility throughout the gestation period.
The aim of this study is thus to evaluate, *in vitro*, the spontaneous contractility of bovine uterus at different stages of pregnancy (from the first at 30 days to the last at 270 days) in order to understand the physiology of this organ, and provide important background information for the effect of drugs on the bovine pregnant uterus.

MATERIALS AND METHODS

Sixty-three pregnant uteri were obtained from cows slaughtered at a local abattoir. All uteri were found to be free from diseases. Pregnancy has been divided into 9 periods of 30 days each (from 0-30 days to 240-270 days) and 7 strips for each stage of pregnancy (total 63 strips), were considered in our study.
Before stunning, blood samples were collected from the coccygeal vein of each cow in vacutainer glass tubes. Once in the laboratory, blood was centrifuged at 1620g for 10 min at 4°C. The sera were subsequently frozen at - 20°C for later analysis of P_4 which was conducted with a competitive immunoenzymatic colorimetric method (Progesterone EIA WELL, Radim S.p.A., Italy). The cross-reactions between P_4 and steroid hormones were reported as follows: P_4 100%; 11-α OH-P_4 18%; 17-α OH-P_4 16%; 20-α OH-P_4 1%; estradiol $<1\times10^{-2}$%; testosterone $<1\times10^{-2}$%; cortisol $<1\times10^{-3}$%; cholesterol $<1\times10^{-3}$%. The detection limit of the assay was 0.16 nmol/l. The intra-assay and inter-assay precision had coefficients of variation of 2.9% and 4.8%, respectively.
Estradiol-17β (E_2) concentration was determined by an immunoenzymatic method (Estradiol ELISA, Dia. Metra S.r.l, Italy). The cross-reactions of the antibodies are reported as follows: E_2: 100%; Estrone 2%; Estriol 0.39%; Testosterone 0.02%; Cortisol $<7\times10^{-3}$%; Progesterone $<3\times10^{-4}$%; Dhea-s $<1\times10^{-4}$%. The lowest detectable concentration was 15 pg/mL at the 95% confidence interval.
Gestational age (in days) was recognised by *ante* and *post-mortem* examination.
Ante-mortem, pregnancy status was diagnosed, for the first trimester, by clinical examination associated with B-mode ultrasonography (SonoSite MicroMaxx Bothell WA, USA with a 7.5 MHz linear probe), as summarized by Hughes and Davies[10].
Post-mortem, the animals' genital tract was visually examined to confirm the stage of pregnancy (in days) and to identify the gestational age (for those in the last six months). Gestational age was found by measuring the pregnant horn and crown-rump length[11].
After slaughter, the uteri were collected in about 20±10 min. From each uterus, a single circular portion of the middle part of the gravid horn was excised and immediately placed in a flask containing pre-refrigerated Krebs solution (NaCl 113 mM, KCl 4.8 mM, $CaCl_2$ $2H_2O$ 2.2 mM, $MgSO_4$ 1.2 mM, NaH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 5.5 mM, sodium-ascorbate 5.5 mM), which was prepared daily. The flask was then immediately transported (in 15±5 min) to the laboratory in an insulated box. From each circular portion, full-thickness (10-mm in length and 3-mm in width) uterine strips of each preparation were cut between two rows of endometrial caruncles and parallel to longitudinal muscle fibres.
The strips were immediately placed in a jacketed organ bath (mod. 4050 Ugo Basile, Milan, Italy) containing 10 ml of Krebs solution and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was kept at 7.4, and the temperature was maintained at 37°C. A silk thread was used to attach the myometrial strips to a fixed hook and an isometric force displacement transducer (FORT25; AD Instruments, Castle Hill, NSW, Australia). Contractile activities were recorded using a PowerLab 4/35 (AD Instruments acquisition software). During the first 60 min, the strips were allowed to stabilize in the organ baths without applying tension. Subsequently, the strips were allowed to equilibrate under a constant tension of 2 g for about 30 min. After the equilibration period, carbachol (10^{-5} M) (Sigma-Aldrich, Milano, Italy), the esterified form of acetylcholine, which has a selective and prolonged contractant effect, was added in a cuvette. This dose, dissolved in Krebs solution, was subsequently removed by washing (*wash-out*), followed by a period of 30 min or more, needed for the strip to return to baseline. Subsequently, a second dose of carbachol (10^{-5} M) was added to the cuvette and its effects were compared to those obtained in the previous administration[12].
In the presence of a repeatable response with a deviation ≤20%, calculated by the formula: $(Value_{Maximum}\text{-}Value_{minimun}/Value_{Maximum})*100$, we proceeded to test the experimental protocol, otherwise, again after 30 min, a third dose of carbachol was administered at the same concentration (10^{-5} M). If this final administration of carbachol was not repeatable with at least one of the previous doses, the strip was discarded from the experiment[12].
Thereafter, regular spontaneous oscillatory contractions were recorded for 30 min.
Finally, to evaluate the functionality of the strip throughout the experiment, the registration period, was followed by *wash-out* and by a dose of carbachol (10^{-5} M). The response of the strip had to be repeatable (within20%) compared to that for the previous administration[12].
Amplitude, frequency and area under the curve (AUC) of contractions for each strip were determined by analysing the sequence corresponding to the last 30 min of contraction, from the recording section.
For motility studies, all amplitude, frequency and AUC values were expressed as mean ± SEM and were subjected to statistical analysis by SPSS® Statistics 19 (IBM®, NY).
Differences among groups of bovines in the same month of pregnancy were compared using a one-way ANOVA and LSD post hoc test.
The values were considered significant for $p < 0.01$.
For serum analysis, all values were expressed as Mean ± S.D. Data were analyzed by GLM for repeated measurements, and a post hoc LSD test was applied.
A $p < 0.05$ was considered statistically significant.

RESULTS

Spontaneous uterine contractility was observed in 57 strips out of 63 uteri collected. Six strips showing no spontaneous or comparable responses to carbachol (10^{-5} M) were discarded.
All strips selected showed regular spontaneous contractions after 30 min of constant tension. The last 10 min of contrac-

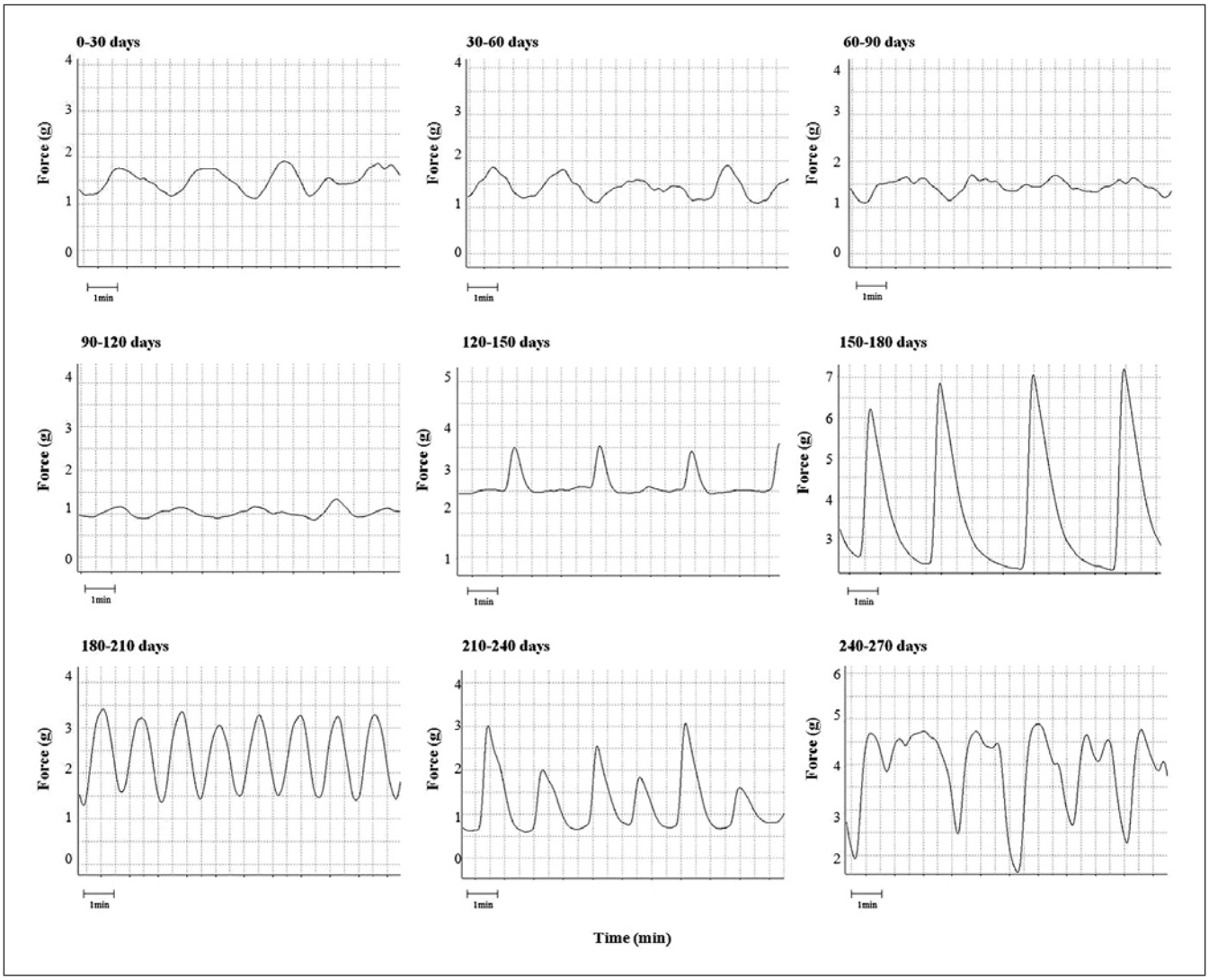

Figure 1 - Representative tracing of spontaneous uterine contractility, during different gestation periods (days) in pregnant cows. The abscissa shows cumulative time (10 min) after the beginning of the session. The ordinate shows the force (tension) in grams.

tion recording, for all stages of pregnancy, are reported in Fig. 1, such as a representative tracings.

The mean amplitude, frequency and AUC values ± SEM of spontaneous contractile activity (30 min) in pregnant bovine, during the experimental period, are reported in Fig. 2, 3 and 4.

Contractility proved extremely limited in early pregnancy (from 0-30 to 90-120 days) and even more so between 120-150 and 180-210 days, reaching maximum force (amplitude) of contraction between 150-180 days. After this period, it decreases from days 180- to 210 and then rises again from days 240- to 270 (Fig. 1 and 2). The contractility detected in the latter two periods is very unusual: between 210-240 days, it is made up of alternating waves with low and high strength, while the period from 240-270 days is characterized by contractures (high-amplitude, low-frequency and long-lasting) (Fig. 1).

Mean contraction frequency was significantly different in the nine groups and lowest again at 90-120 days, while it increased during the latter periods tested in our study (from 180-210 days to 240-270 days) with the highest values coming at 180-210 days (Figs. 1 and 3).

By contrast, AUC follows the same trend as that described for the amplitude of contraction (Fig. 4).

Serum analyses were in accordance with a previous study[13]. The results showed a higher Estradiol-17β/Progesterone ratio between 90-120 days and 150-180 days (Table 1).

DISCUSSION

Many substances (hormones and neurotransmitters) can modulate uterine contractility in non-pregnant and pregnant cows. However, although spontaneous and induced contractility have been well analyzed in non-pregnant cows (follicular and luteal phases, healthy subjects and animals with endometritis)[9,12,14,15,16], few studies have reported results for pregnant cows[4,5,7,8,17,18]. Indeed, to our knowledge, this is the first study that has evaluated the spontaneous contractility of bovine uterus, during every single month of pregnancy (from the first 30 days to 270 days).

Our results showed that, in all of the periods analyzed, there was spontaneous uterine contractility, with contractions varying in strength and duration. Indeed, myometrial activity proved extremely limited in early pregnancy (up to 120 days) and even more in mid-pregnancy (from 120-150 to 180-210 days), achieving of its most impressive contractions at 150-180 days. After this period, uterine contractility de-

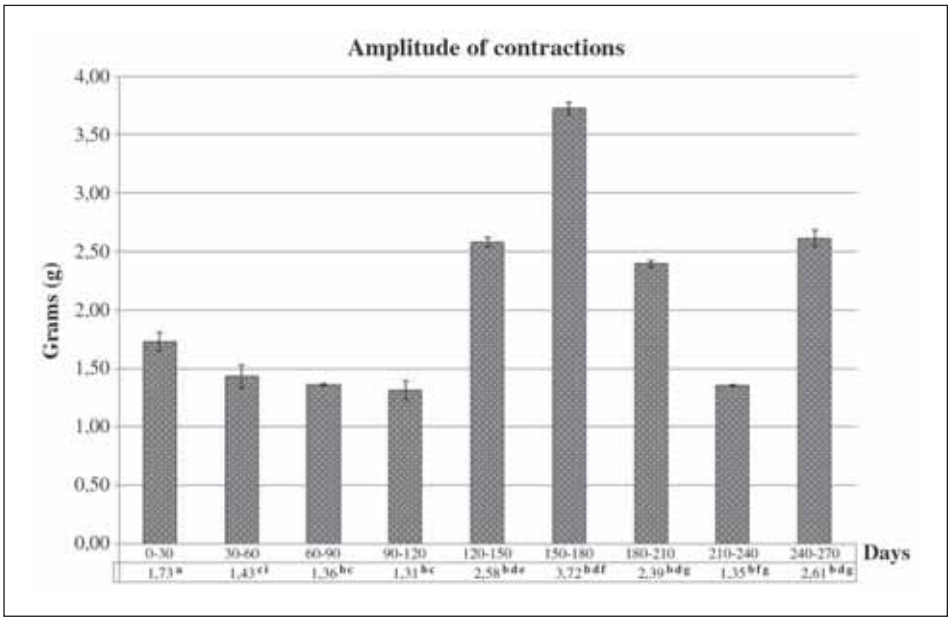

Figure 2 - Amplitude of uterus spontaneous contractile activity (30 min) in pregnant bovine in nine different gestation periods (days). Data are expressed as mean ± SEM.
In line: a, i: p <0.01; a, b; c, d; e, f; f, g: p <0.001.

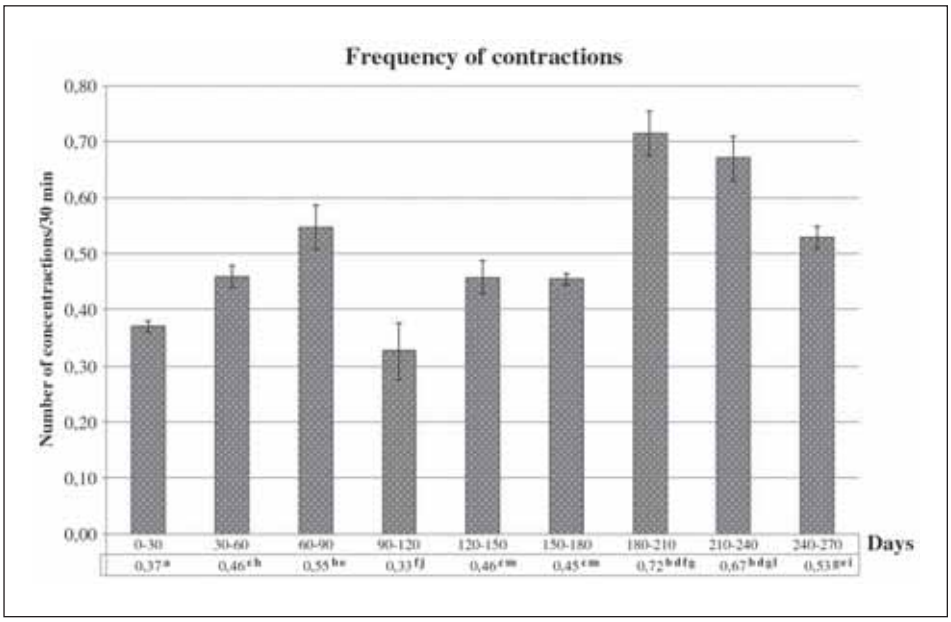

Figure 3 - Frequency of uterus spontaneous contractile activity (30 min) in pregnant bovine in nine different gestation periods (days). Data are expressed as mean ± SEM.
In line: a, i; h, j; e, l; j, m: p <0.01; a, b; c, d; e, f; j, g: p <0.001.

creases and then rises again to hight values at 240-270 days. Hence, in accordance with previous studies[5,7,8,9], these results support the hypothesis that the bovine pregnant uterus is not completely quiescent.

During pregnancy, due to the presence of P_4, we would expect a uterus with slow contractility. In our results, it this occurs in early pregnancy (up to 120 days) and at 210-240 days, but neither in mid-pregnancy (between 120 and 210 days) nor at 240-270 days.

This difference in contractility may well be due to local and systemic hormone or neurotransmitter concentrations, during the different stages of pregnancy.

In this regard, steroid hormones exert overall regulation of myometrial activity and it is generally accepted that estrogen promotes uterine activity whereas P_4 favors uterine quiescence through the stimulation or the reduction of gap-junctions. Indeed, the distribution of these intercellular communications is closely related to hormonal changes (estrogen/P_4 ratio)[14,15]. However, we showed that P_4, Estradiol-17β and Estradiol-17β/P_4 ratio, in peripheral blood, are maintained relatively constant in early-pregnancy and that these values undergo changes from mid-pregnancy. In fact, during the second trimester, Estradiol-17β levels rise slightly from about 90-120 days to 150-180 days, and then drop slightly at around 180-210 days. After this time, Estradiol-17β levels rise again at 240-270 days (Table 1). These results are in agreement with other studies in which steroid hormone concentrations in maternal blood and placental fluids are reported[8,17]. These systemic and local concentrations of steroid hormones are probably essential for implantation and placentation[19] and for adjusting the histotrophic environment necessary for *conceptus* growth and development[20]. This hormonal *milieu* could justify the presence of changes in myometrial contractile activity, during the different stages of bovine pregnancy.

In the presence of a stable Estradiol-17β/P_4 ratio, the contractility of the pregnant uterine horn is very limited. In this trimester of pregnancy, this low contractility could probably allow for complete placental insertion. Instead, when the Estradiol-17β/P_4 ratio starts to increase, there is a slow rise in contractility, that becomes evident only at 120-150 days and reaches its peak at 150-180 days. Indeed, in addition to depolarizing membranes, estrogens favour the up-regulation of oxytocin receptors[21]. In fact, it has been shown that, around days 60-90 of pregnancy, there is an increase in the number of oxytocin receptors in bovine myometrium, which *plateaus* at 150-180 days and remains unchanged until a few days before calving[22].

This transitory increase in contractility is physiological and promotes placental exchanges and placental function, that are very important to foetal development. However, it could be extremely dangerous, because any stress or infection in

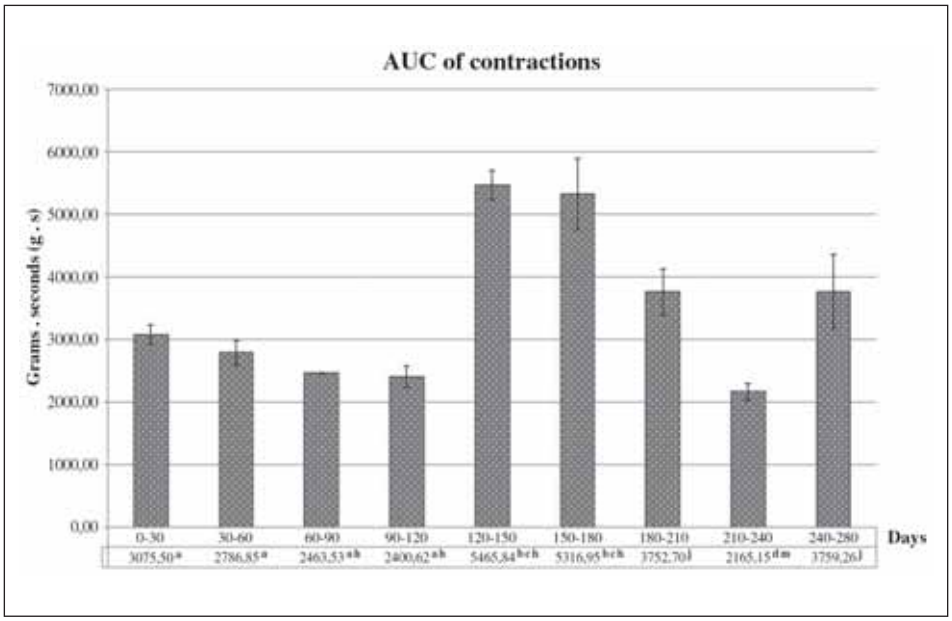

Figure 4 - Area under the curve (AUC) of uterus spontaneous contractile activity (30 min) in pregnant bovines in nine different gestation periods (days). Data are expressed as mean ± SEM. In line: h, j; j, m: p <0.01; a, b; c, d: p <0.001.

Table 1 - Progesterone (ng/L) and Estradiol-17β (ng/L) levels in maternal blood and their ratio (ng/L). Data are expressed as mean ± DS.

Gestational stage	Maternal blood		
	Progesterone (ng/L)	Estradiol-17β (ng/mL)	Estradiol-17β/ Progesterone (ng/mL)
0-30 days	5.0 ± 7.45 ^{A}	0.040 ± 0.04 ^{A}	0.008
30-60 days	6.0 ± 7.88	0.033 ± 0.04 ^{A}	0.006
60 - 90 days	6.40 ± 7.23	0.037 ± 0.04 ^{A}	0.006
90 - 120 days	8 ± 6.10 ^{B}	0.133 ± 0.11 ^{B}	0.017
120 - 150 days	7.3 ± 5.34	0.108 ± 0.10	0.015
150 - 180 days	6.85 ± 8.72 ^{B}	0.122 ± 0.06 ^{B}	0.020
180 - 210 days	7.12 ± 5.05 ^{B}	0.072 ± 0.06	0.010
210 - 240 days	7.56 ± 7.53	0.075 ± 0.05	0.010
240 - 270 days	8.10 ± 6.81 ^{B}	0.096 ± 0.07	0.012
In column: A, B: p <0.05.			

mid-pregnancy could induce a further increase in pro-inflammatory cytokines[23] and contractile activity, responsible for abortion. Indeed, the greatest number of abortions caused by bacteria, viruses, protozoa and fungi, occurs exactly during mid-pregnancy[24].

After this time, we found a decrease in contractility, dropping slightly from 180-210 days to 210-240 days. Moreover, around 210-240 days, the contractility is very unusual, made up of alternating waves of low and high strength. This contractile activity is probably only a transitory phase, during which the uterus becomes less responsive to endogenous and exogenous hormonal rates, as reported by Minoia et al.[7].

Instead, the period from 240-270 days is characterized by a contractile activity called "contracture". These are long-lasting contractions, with high amplitude and low frequency, which have been well described by Kündig et al.[5]. This contractile activity is probably functional to placental "maturation", a prerequisite for the sub-partial detachment and release of the foetal membrane[25]. However, it is possible to believe that the increase in contractility in the later stages may be due to the gradual increase in alpha$_2$-adrenergic receptors. Indeed, the bovine uterus is extremely rich in alpha$_2$-adrenoreceptors with excitatory activity, whose concentration rises in the presence of high estrogen level[26,27]. This is extremely important from a clinical point of view, and particularly in the choice of sedative in pregnant cattle surgery. For example, the use of xylazine, a known alpha$_2$-agonist, could increase uterine contractility and, subsequently, increase the risk of abortion.

CONCLUSIONS

In conclusion, our study is the first work to show contractility in a pregnant uterus in the different months of gestation. It showed variable contractility during pregnancy, with the lowest values at 90-120 days and the highest at 150-180 days.

These results explain the importance of several factors, in particularly steroid hormones, in pregnancy (P_4 in early-pregnancy and estrogens in late-pregnancy) and provide a new key to understanding the causes of abortion.

This study serves as an aid to the clinician in the choice of sedative to use for surgery in pregnant subjects. Further studies are, however, necessary to clarify the relationship between the contractility detected in this study and the concentration of alpha$_2$-adrenoreceptors.

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The effect of high dietary zinc oxide supplementation and farming conditions on productive performances of post-weaning piglets

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SUMMARY

Zinc supplementation is recommended to prevent scours and improve weight gain of weaned piglets but the effects are variable and they may be influenced by many housing and weaning techniques, the hygienic status of the housing and climatic factors. The aim of this study was to compare the effects of zinc oxide supplementation at a dose of 2400 mg/kg feed, with different periods of administration and housing conditions, on growth performance and mortality in the post-weaning period of piglets. A total of 360 piglets, in two farms with different housing conditions were assigned to three treatments to estimate the effect of zinc oxide supplementation in the post-weaning period: the Zn21 group was offered a diet supplemented with 2400 mg/kg of zinc oxide for the first 21 days of the post-weaning period; the Zn56 group was given a diet supplemented with 2400 mg/kg of zinc oxide for 56 days; the Control group had a diet without any zinc supplementation. Live weight, feed consumption and mortality rate were recorded. Data showed that Zn diet administrated for 21 days in piglets did not overall improve growth performance if compared with control group. Furthermore, supplementation for 56 days reduced live weight. The productive performances were also influenced by the housing conditions. Concluding, we can confirm that the first days of the after weaning period are the most delicate for piglets. In this phase their performances were more affected by the housing conditions than the diet.

KEY WORDS

Piglets, zinc oxide, weaning, live weight gain, housing condition.

INTRODUCTION

Research on the use of growth promoters other than antimicrobial molecules has increased significantly after the Regulation (EC) No 1831/2003 of the European Union[1] which directed that antibiotics were no longer authorised as feed additives. Zinc supplementation has been recommended since the 1980s to prevent scours and improve weight gain of weaning piglets[2], and different concentrations have been tested and used[3]. Treatments with a low concentration (80 mg/kg of feed) of zinc were found to not significantly affect average daily gain and feed intake of piglets in the first five weeks after weaning[4], while better performance was obtained using concentrations between 2000 and 4000 mg/kg of feed[5]. In the European Union[6], the use of zinc oxide is authorised at a maximum concentration of 150 mg/kg of feed, whereas much higher concentrations, from 2000 to 3000 mg/kg, are admitted only as a pharmacological treatment under prescription by a veterinarian. Nevertheless, zinc oxide supplementation at high dosage is a common practice for controlling the high mortality of piglets during weaning[7] and even if the supplementation period is commonly limited to 21 days, there is disagreement about the effects of dose and duration of Zn supplementation on mortality and growth performance of weaning piglets[8,9].

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The use of zinc oxide at high concentration for extended periods raises concerns about environmental pollution[9], as the exceeding amount passes in the excreta increasing this risk[10] and its use should be limited. Moreover, the effects of zinc oxide supplementation are variable and they may be influenced by many housing and weaning techniques[7], the hygienic status of the housing, and climatic factors.
The aim of this study was to compare the effects of zinc oxide supplementation at a dose of 2400 mg/kg of feed, with different periods of administration and housing conditions, on growth performance and mortality in the post-weaning period of piglets.

MATERIALS AND METHODS

The experiments described in this article have been carried out in accordance with EU Directive for Animal Experiments[11].

Farms, housing and pigs

The research was carried out in two commercial growing farms located in Sardinia, Italy, which were part of the same integrated system for the intensive production of pig meat, organized in rearing, growing and fattening farms. The two farms, here indicated as Farm A and Farm B, had different housing, management and animal density conditions, which are summarized in Table 1. They were 50 km far from each other, with Farms A and B being 23 and 36 km, respectively, from the rearing farm where the piglets were born. Three hundred and sixty castrated male piglets

Table 1 - Comparative characteristics of the two farms.

Characteristics	Farm	
	A	B
Altitude (m above sea level)	60	200
Building	two doors but no window	one door and uninterrupted windows 2 m above the floor
Pens		
placing	double row of 12 pens and a walkway in the middle	double row of 10 pens and a walkway in the middle
floor	inclined continuous concrete with a manuring ditch on the opposite side of the walkway	concrete slotted floor
dividing barriers	cement-plastered concrete walls with a height of 1 m	removable iron fences with a height of 1 m
measures (m)	2×2	2×2.5 m (the longest side bordering the walkway)
animal density[1] (m^2/piglets)	0.26	0.33
Manger and water dispenser	manger of concrete cement on the side bordering the walkway, two stainless steel nipple water dispensers on the side contiguous the manuring ditch	a steel alloy feeding manger on the side bordering the walkway and two stainless steel nipple water dispensers on the opposite side
Conditioning air system (cooling and heating)	Not present	Not present
Mean mortality[#] (%)	4.3 - 4.8	3.8 - 4.2

[1] Calculated on the basis of 15 piglets per each pen. [#] Recorded in the previous three years.

of the same genotype (crossbred Pietrain × PIC) were randomly selected from the same batch, weaned at approximately 28 days of age and randomly assigned to the two farms (180 at Farm A and 180 at Farm B) and three different treatment groups.

Zinc oxide treatment and feeding

At each of the two farms, on the day of arrival, piglets were randomly assigned to one of the three different feeding treatments. Piglets assigned to the control group (C) received a starter diet for 21 d and a transition diet for the following 35 d (Table 2), without any zinc oxide supplementation; the Zn21 group was fed the same diets as the C, supplemented with 2400 mg of Zn oxide for kg of feed for the first 21 days (D0-D21); the Zn56 was fed the same diets as the C, supplemented with 2400 mg of Zn oxide for kg of feed for the entire trial (D0-D56). The zinc oxide (Zincofarm G, Sintofarm SpA, Guastalla, Italy) powder has been added to the diet by the feed producer. Control, Zn21 and Zn56 feed were produced starting from the same batch for the whole trial. All groups were fed *ad libitum* and water was freely available from the nipple water dispensers.

Two representative samples for the starter and two for the transition ration were collected, with replications for each farm with a total of 16 samples. Samples were analysed for their proximate composition according to Association of Official Analytical Chemists[12] and the metabolizable energy of the feeds (Table 2) was computed from ingredient composition using tabulated values.[13]

For each treatment, groups made up of 15 piglets were housed in different pens. At each farm, pens were adjacent and located in the middle of the housing rooms with the following sequence: C, Zn21, Zn56, C, Zn21, Zn56 and so on. In summary, a total of 24 different pens equally distributed in two different farms (12 pens at A and 12 at B) and three different feeding treatments (8 pens for C, 8 pens for Zn21, 8 pens for Zn56) were investigated.

Measurement and records

On the day of arrival at the growing farm, the same day of weaning (day 0, D0), piglets were assigned to the pens (15 piglets per each pen). Each pen has been considered as a single experimental unit. Piglets have been individually weighed by the use of a 100x70 cm portable electronic four cells scale (D400, Coop. Bilanciai, Italy). The sum of all the weights of the piglets in a pen was used as the weight of the experimental unit. The subjects were weighed again after 21 days, D21, and after 56 days, D56. Experimental units remained the same for the whole trial, but the calculation of average daily gain (ADG) varied depending on the number of the piglets in the pen, as in some of the pens some piglets died on D0-D21 growing period. ADG was computed as the ratio between the difference of two consecutive weighings and the number of days.

Feed was administrated to piglets on concrete feeders fulfilled every day by adding feed to the remaining one from the previous day, and consumption was obtained calculating the difference between the amount of diets offered to each experimental unit each day and the quantity of feed which remained in the feeder at D21 and D56. Feed efficiency was computed as the ratio between ADG and feed consumption.

Daily maximum and minimum temperature and daily maximum and minimum relative humidity were recorded using a digital thermo-hygrometer (KT-304, KTI Ketai Instrument, China) which was suspended in the middle of the building at 1 m above the floor level. The number of dead piglets was recorded daily.

Furthermore, feeding costs were determined considering the local market prices of the diets ingredients, including zinc oxide, and feed consumption. Gross income was calculated as the

Table 2 - Ingredients, metabolizable energy and chemical composition of basal diets.

Item	Starter diet (D0-D21)	Transition diet (D22-D56)
Ingredient (g/kg)		
Corn	297	286
Wheat	243	271
Barley	165	211
Potato (protein concentrate)	88	21
Soybean meal	–	116
Wheat gluten	21	16
Soybean oil	27	16
Whey	141	36
L-Threonine	1	2
Tryptophan	5	–
L-Lysine hydrochloride	5	6
DL-Methionine	1	1
Choline	1	1
Sodium chloride	2	4
Calcium carbonate	3	7
Dicalcium phosphate	3	6
Calculated metabolizable energy (MJ/kg)[1]	14.36	13.94
Analysed composition (g/kg)		
Crude protein	175	168
Crude fat	55	50
Ashes	58	57
Crude fibre	28	35
Other chemical constituents[#]		
Lysine (g/kg)	1	1
Vitamin A (IU/kg)	15000	15000
Vitamin D3 (IU/kg)	2000	2000
Vitamin E (mg/kg)	200	200
3-phytase (EC 3.1.3.8) (FTU/kg)	500	500
Apramicine (IU/kg)	100	–

D0, D21, D22 and D56: day 0, 21, 22 and 56 of the growing period of piglets. [1] computed from ingredient composition using tabulated values given by Sauvant et al.[12]. [#] added in the feed but not analysed.

difference between the selling price of the whole weight gain of piglets and feeding costs, using the following equation:

[selling price per kg × (whole weight of piglets on D56 - whole weight of piglets on D0)] - (cost of feeding per kg × whole amount of feed offered the piglets in the period D0-D56).

The selling price per kg was calculated on the basis of live weight of piglets at D56 and average prices of one of the most important national market for pig meat (Modena, Italy).

Statistical Analysis

Data regarding live weight of piglets, average daily gain, feed consumption and feed efficiency were analysed by means of the mixed effect model which considered as fixed effects the feeding diet (3 levels: C, Zn21 and Zn56), the farm (2 levels: A and B) and the interaction between diet and farm. Multiple comparison of least square means (LSM) was performed with the Bonferroni method.

Mortality was analysed by means of Fisher's exact on the basis of the diet, farm and weighing session. All statistical analyses were performed using the using the SAS software (version 9.3, SAS Institute Inc., Cary, NC) and model effects were declared significant at $P<0.05$.

RESULTS

In order to provide a general view of climatic conditions between the farms, data regarding temperature and humidity are reported in Table 3. Both parameters recorded the highest values at Farm A.

Results regarding mortality according to the diet, the farm and the weighing session are reported in Table 4. In farm A, 6 piglets died during the D0-D21 growing period, 2 (1.11% of the whole farm A), 0 and 4 (2.22%) for C, Zn21 and Zn56 diet, respectively. In the farm B 7 piglets died during D0-D21, 2 (1.11% of the whole farm B) in C, 1 (0.56%) in Zn21 and 4 (2.22%) in Zn56. There was no significant difference for any of the effects. For all the dead piglets a post-mortem examination showed they died of a bacterial enteritis.

Results regarding live weight and average daily weight gain of piglets are shown in Table 5. Live weights were influenced on D21 at $P = 0.001$ by the farm, with the highest values registered at Farm B, and on D56 by the diet (at $P =$

Table 3 - Least square means ± standard deviation of minimum and maximum temperature (T) and relative humidity (RH) throughout the 56 days of the trial according to the farm.

	Farm	
	A	B
T min (° C)	19.64 ±3.27	17.76 ±3.05
T max (° C)	24.95 ±3.22	23.14 ±3.56
RH min	65.67 ±9.82	58.75 ±7.58
RH max	86.44 ±6.55	76.98 ±8.09

Table 4 - Mortality of piglets according to the diet, the farm and the weighing sessions.

		Diet			Farm	
		C	Zn21	Zn56	A	B
Piglets	n	120	120	120	180	180
Dead D0-D21	n	4	1	8	6	7
Dead D22-D56	n	0	0	0	0	0
Alive on D56	n	116	119	112	174	173
Test		Fisher's exact			Fisher's exact	
P-value		0.056			1.000	

D0, D21, D22 and D56: day 0, 21, 22 and 56 of the growing period of piglets. C: no zinc supplementation, control group; Zn21: 2400 mg/kg of zinc oxide from D0 to D21; Zn56: 2400 mg/kg of zinc oxide from D0 to D56.

Table 5 - LS means of live weight and average daily weight gain of piglets according to the diet and the farm.

	Diet			SEM	Farm		SEM	Effect and P-value		
n#	C 8	Zn21 8	Zn56 8		A 12	B 12		D	F	D×F
Live weight (kg)[1]										
D0	8.9	8.8	8.7	0.05	8.8	8.78	0.05	0.329	0.807	0.070
D21	15.2	14.4	14.2	0.16	13.8^{A}	15.4^{B}	0.16	0.095	0.001	0.323
D56	37.3^{b}	36.5^{b}	34.4^{a}	0.40	33.4^{A}	38.7^{B}	0.41	0.024	0.001	0.008
Average daily weight gain (kg)[1]										
D0-D21	0.283	0.257	0.247	0.01	0.228^{A}	0.297^{B}	0.01	0.062	0.001	0.072
D22-D56	0.632^{B}	0.631^{B}	0.589^{A}	0.02	0.590^{A}	0.667^{B}	0.02	0.005	0.001	0.002

[1] Data are the mean value referred to the individual piglets.
[#] n is referred to the observations of the individual pen.
Means in the same row with different superscript are significantly different according to the diet or the farm. Capital letters indicate P<0.01; lower P<0.05.
SEM: standard error of the means.
D0, D21 and D56: day 0, 21 and 56 of the growing period of piglets.
C: no zinc supplementation, control group; Zn21: 2400 mg/kg of zinc oxide from D0 to D21; Zn56: 2400 mg/kg of zinc oxide from D0 to D56.
D: Diet; F: Farm; D×F: interaction Diet × Farm.

0.024), the farm (P = 0.001) and the interaction (P = 0.008). Regarding this latter effect, lowest values have been registered for group Zn56 at Farm A (data not shown in tables). Average daily weight gain was influenced by the diet in the period D22-D56, and by the farm in both the periods D0-D21 and D22-D56. The interaction between farm and diet affected ADG in the period D22-D56 and the highest values were registered for C and Zn21 groups at Farm B and the lowest in the Zn56 group at Farm A (data not shown in tables).

Data regarding feed consumption and feed efficiency are shown in Table 6. Feed consumption has been affected by the Farm during the starter (P = 0.033) and the transition period (P = 0.001), whereas feed efficiency only during the starter period (P = 0.046). Farm B registered the highest values for both parameters. As regards the interaction between farm and diet, only feed consumption (P = 0.032) in the period D22-D56 showed significant differences, with the highest values registered for C and Zn21 at Farm B compared to Zn56 group of Farm A (data not shown in tables).

Even if a statistical analysis was not performed on economic parameters, these are presented in the form of percentage differences: total gross income registered for piglets belonging to C (5,881 €) and Zn21 treatments (5,979 €) were respectively 9.8% and 8.0% higher than the value regarding Zn56 (5,444 €); data registered at Farm B was 21.9% higher than Farm A (9,507 and 7,798 €, respectively).

DISCUSSION

On the basis of the comparative data about buildings and facilities and the climatic conditions recorded at the farms, the main factors defining the difference between the two farm could be considered the flooring (slotted at Farm B), space allowance (higher at Farm B) and temperature and humidity (lower at Farm B). Even if many differences were evidenced in housing and feeding conditions, results regarding mortality were not affected by the diet and farm effects and the overall number of dead piglets was similar to that normally

Table 6 - LS means of feed consumption and feed efficiency according to the diet and the farm.

	Diet			SEM	Farm		SEM	Effect and P-value		
n#	C 8	Zn21 8	Zn56 8		A 12	B 12		D	F	D×F
Feed consumption (kg)[1]										
Starter period (D0-D21)	0.437	0.428	0.426	0.01	0.396^{a}	0.447^{b}	0.01	0.773	0.033	0.134
Transition period (D22-D56)	1.088	1.066	1.059	0.01	0.987^{A}	1.142^{B}	0.01	0.865	0.001	0.032
Feed efficiency[2] (gain:feed)[1]										
Starter period (D0-D21)	0.680	0.630	0.608	0.01	0.578^{a}	0.708^{b}	0.01	0.643	0.046	0.233
Transition period (D22-D56)	0.581	0.592	0.546	0.01	0.568	0.584	0.01	0.657	0.102	0.082

[1] Data are the mean value referred to the individual piglets.
[2] Ratio between average daily weight gain and feed consumption.
[#] n is referred to the observations of the individual pen.
Means in the same row with different superscript are significantly different according to the farm. Capital letters indicate P<0.01; lower P<0.05.
SEM: standard error of the means.
D0, D21 and D56: day 0, 21 and 56 of the growing period of piglets.
C: no zinc supplementation, control group; Zn21: 2400 mg/kg of zinc oxide from D0 to D21; Zn56: 2400 mg/kg of zinc oxide from D0 to D56.
D: Diet; F: Farm; D×F: interaction Diet × Farm.

registered in the previous production cycles and years. On the other hand, it should be evidenced that the P value registered for the diet effect (0.056) tended to be closer to the significance limit.

On the whole, data regarding live weight, weight gain, feed consumption and feed efficiency showed that the influences on these traits were caused above all from the different levels of the building and housing conditions. These results might have been affected by the better environmental conditions (temperature and humidity), flooring and space-allowance at Farm B, since mean individual space-allowance was 0.33 m^2/piglet at Farm B and 0.26 at Farm A. This is consistent with the study by Slade et al.[14], which demonstrates that zinc oxide supplementation had different effects in dissimilar rearing systems.

The absence of statistical differences in growth rate and daily gain between the piglets belonging to the control group and those supplemented with zinc for 21 days was in accordance with a previous research[9]. Conversely, the groups fed with a zinc supplemented diet for the overall post-weaning period, Zn56, showed the lowest values of live weight and weight gain. The study by Reynolds et al.[15] has shown a significant reduced intake of the zinc oxide supplemented feed by piglets at first experience, because of reduced palatability. In the present study, the highest weight gain in the groups Zn21 and C was not due to a difference in feed intake, because the average daily amount of diets consumed by the piglets and the feed efficiency were similar among the groups. The beneficial effects of the inclusion of zinc in diet for weaning piglets has been ascribed to the reduction of diarrhoea and inhibition of histamine release from mast cells[16,17] and to a lymphocytosis[8].

Other studies have reported that zinc oxide supplementation at high concentration (2500-3000 mg/kg) might improve productive performance during the post-weaning period[2,18]. Also Hill et al.[5] and Davis et al.[8] record an increased weight gain and feed intake in weaning piglets supplemented with zinc, respectively at 1500 to 2000 and 2500 mg/kg. Lastly, Poulsen[19] reports intermediate results, as a supplementation similar to the one of the present study influences daily gain but not feed intake. On the basis of the present study, the best results were obtained when the duration of supplementation at high concentration in the post weaning period did not exceed three weeks; this could also avoid concerns regarding environmental pollution deriving from zinc accumulation[9,18] and selective effects on gut microbiota[9].

In the present study, the highest differences of gross income were recorded between the farms and the calculation of this parameter could eventually provide to the farmers a direct economic evaluation of the productive performance of the piglets.

CONCLUSIONS

On the basis of the results of this study, it can be concluded that diet supplemented with Zn oxide at the concentration of 2400 mg/kg for 21 days after weaning did not overall improve growth performance of piglets, while supplementation for 56 days reduced live weight. Moreover, despite the satisfactory climatic conditions at the farm with low level of facilities, in a typical Mediterranean environment, productive performances were negatively influenced by the lower housing conditions.

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CEVA

Fetiform teratoma in an Italian-Friesian calf: case report and literature review

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SUMMARY

Introduction - *Fetiform teratoma* is a rare form of teratoma in animals and people that resembles a malformed fetus. This paper describes the first case of highly differentiated extragonadal *fetiform teratoma* with cranial connection in an Italian-Friesian calf.

Case presentation - A 35-day-old male Italian-Friesian calf weighing 55 kg was referred because of a mass localized in the fronto-nasal region. The mass contained two lateral structures of similar size and conformation that were recognized as underdeveloped hind limbs, while at its center there was a small tail. The mass was surgically excised and sent to the pathologist for examination. Gross examination identified two femur-like rudimentary limbs and a sketch of bone located in between, morphologically referable to a rudimentary coxae-like bone. Some mucinous cysts, a virtual body cavity showing adipose and muscular tissues, some cartilaginous nuclei and a coelomatic body cavity were also noted. Histological examination showed differentiation into skin with dermal appendages, hair, adipose tissue, cartilage, bone, lymphoid tissue, neurovascular bundles, and a rudimentary tail. No neural tissue including spinal cord, brain matter, or gonadal differentiation was seen. On the basis of these findings, the mass was diagnosed as a highly differentiated extragonadal *fetiform teratoma*.

Conclusion - *Fetiform teratoma* should be included among differential diagnoses in cases of neonatal malformation in bovine. Analyzing the available literature, the Friesian genetic strain seem to be predisposed to fetal malformation, but a systematic reporting of cases is needed, in order to investigate further the epidemiological, etiological, pathophysiological and therapeutic aspect of this kind of congenital disease.

KEY WORDS

Theriogenology, calves, malformation, *fetiform teratoma*.

BACKGROUND

Fetiform teratoma is a rare form of highly developed mature teratoma that includes one or more components resembling a malformed fetus[1]. Most authors agree that *fetiform teratomas* are highly developed mature teratomas; the natural history of *fetus in fetu*, however, is controversial[1]. *Fetus in fetu* has often been interpreted as a fetus growing with or within its twin. As such, this interpretation assumes a special complication of twinning, one of several grouped under the term parasitic twin. However, classification of similar congenital malformations is difficult because too few cases have been reported in humans and animals to provide the basis for generalization.

In the present paper, we describe the first case of highly differentiated extragonadal *fetiform teratoma* with cranial connection resembling a case of *craniopagus parasiticus* in an Italian-Friesian calf, successfully treated by surgery.

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CASE PRESENTATION

A 35-day-old male Italian-Friesian calf weighing 55 kg was referred because of a mass on the fronto-nasal region. The delivery was without complications and the calf appeared in good condition with otherwise appropriate development of the musculoskeletal system.

The asymmetrical mass was covered with hair and had well-defined margins. The long axis measured 15 cm and the short axis 10 cm. Two lateral structures of similar size and conformation were recognized as underdeveloped hind limbs, while at the center of the mass a small tail was present (Fig. 1A).

Palpation revealed that the mass was not strictly adherent to the underlying tissues while bone structures were clearly palpable in the central area.

Latero-lateral (Fig. 1B) and cranio-ventral X-ray projection revealed the presence of three bony structures: two with vaguely triangular shape and one with a more oval shape, identified as the pelvic portions of the parasitic twin.

Complete blood count (CBC) and the main haematochemical parameters proved to be in the normal ranges. Aliquots of serum were tested for *Neospora caninum* and *Chlamydia* spp. by indirect immunofluorescence antibody tests (IFAT) and for Bovine Viral Diarrhea Virus (BVDV) and Bovine herpe-

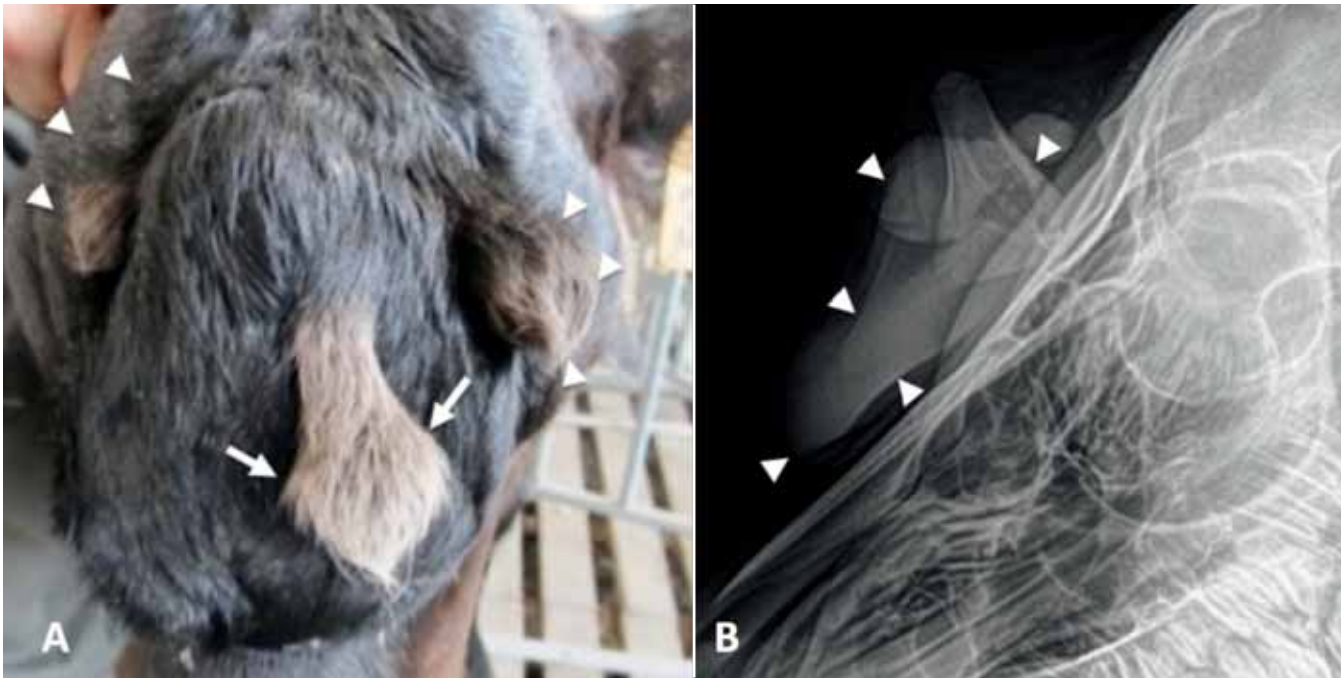

Figure 1 - **A)** Clinical appearance of the mass. Note the underdeveloped hind limbs (arrowheads) and the small tail (arrow). **B)** Latero-lateral X-ray projection of the head. The central structure (arrows) was identified as the pelvic portions of the fetiform teratoma.

svirus-1 (BoHV-1) by enzyme-linked immunosorbent assay (ELISA). RT-PCR and Nested PCR were used to test the bulk milk for BVDV and BoHV-1, respectively. All tests on serum and milk gave negative results.

Cardiac auscultation, electrocardiography, thoracic and abdominal ultrasonography did not reveal any abnormality.

The mass was surgically excised and sent to the pathologist for examination (Fig. 2).

The calf was discharged 11 days after surgery and eight months later was still in good condition as a normal subject.

On gross examination, the well-circumscribed mass excised from the cranial region showed on bisection two rudimentary limbs, each containing an incomplete long bone resembling a femur, and a sketch of bone located between the two appendices, morphologically referable to a rudimentary coxae-like bone (Fig. 3). Inside the excised mass, some small cysts filled with mucinous material were also seen, protruding within a virtual body cavity, whose cut section showed adipose and muscular tissues, and some cartilaginous nuclei that resemble other sketches of bone delimitating a coelomatic body cavity. Multiple cuts did not reveal any axial skeleton or cephalic differentiation. Multiple gross sections, confirmed by the histological examination of the different portions of the mass, showed differentiation into skin with dermal appendages, hair, adipose tissue, cartilage, bone, lymphoid tissue, neurovascular bundles, and rudimentary

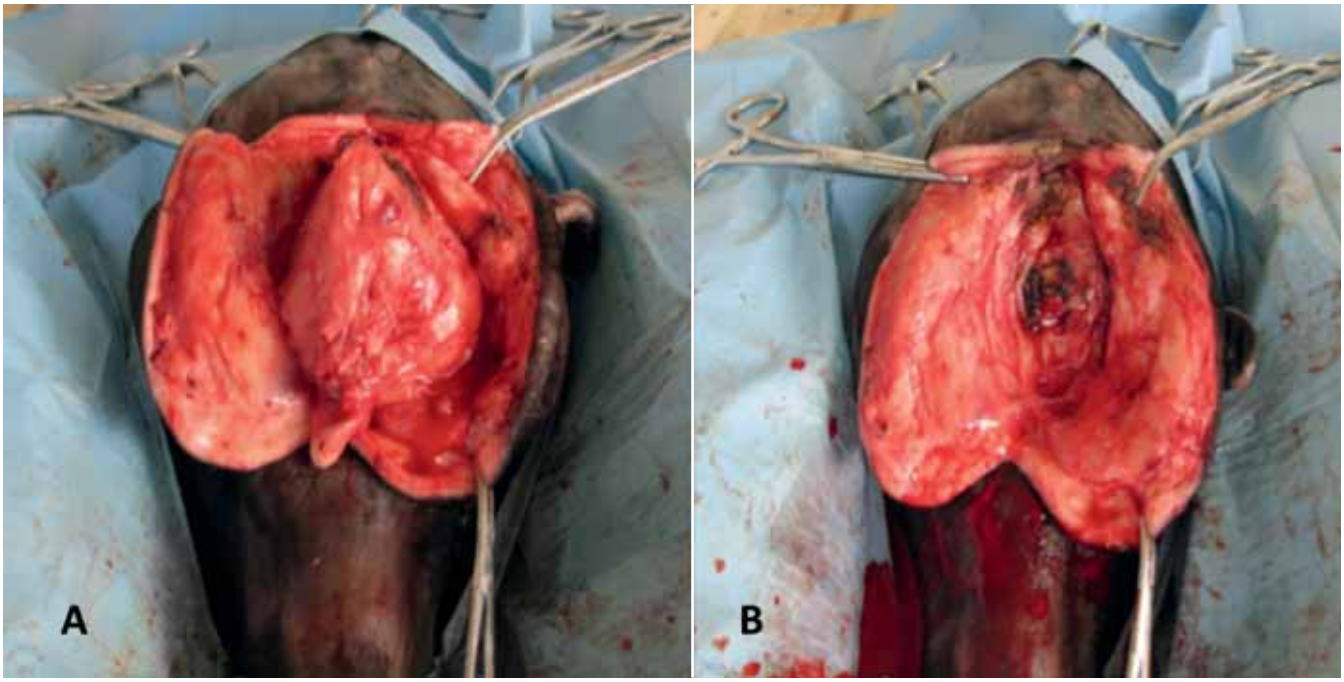

Figure 2 - Intraoperative picture of the surgery: **A)** before and **B)** after removal of the bony mass.

Figure 3 - Sketch of bone, found inside the excised mass, morphologically referable to a rudimentary coxae-like bone.

tail. No neural tissue including spinal cord, brain matter, or gonadal differentiation was seen. On the basis of these findings, the mass was diagnosed as a highly differentiated extragonadal *fetiform teratoma*.

DISCUSSION

Teratomas are embryonal neoplasms composed of tissue derived from all three germ layers. They can be extragonadal or gonadal and arise from primordial germ cells that may become stranded during their migration, coming to rest at extragonadal sites. *Fetiform teratomas* should not be confused with *fetus in fetu*, which is invariably associated with anencephaly and achardia[2,3,4]; difference in the origin of the two has been described in the literature[3]. Unlike classical teratomas, *fetiform teratomas* have complex tissue differentiation/organization and organoid differentiation. Usually the caudal development is better than the cephalic one, as in the present case, which entirely lacks cephalic differentiation. Limb formation is seen more often, while visceral organ tissue and skeletal muscle are inconspicuous or absent, as in this case.

In our case, on the basis of his tissue differentiation and the absence of a head or central and peripheral nervous system, the fetus-like structure may be classified as *fetiform teratoma*, a rare form of mature teratoma that include one or more components resembling a malformed fetus. This teratoma differs from "*fetus in fetu*" because it appears to contain complete organ systems, even major body parts such as the torso, tail, and limbs. *Fetus in fetu* differs from *fetiform teratoma* in having an apparent spine[1].

In our case, surgery was performed successfully and no other abnormalities were detected on the autosite.

Although we cannot establish a breed predisposition, it is interesting to note that most (4 out of 5) cases of parasitic twins reported in bovine have occurred in the Friesian genetic strain[5,6,7,8,9].

There is a dearth of epidemiologic, clinical and pathological information about these congenital malformations because the heterogeneous terminology can cause confusion and also because abnormalities tend to be underreported. Systematic reporting of cases of fetal malformation should be encouraged, in order to provide the basis for further investigation of the epidemiological, etiological, pathophysiological and therapeutic aspects of this kind of congenital disease.

ACKNOWLEDGEMENTS

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UNA FINESTRA SULLE AZIENDE

Come possiamo ridurre e limitare le perdite economiche legate al caldo?

Lo stress da caldo può avere un notevole impatto sulle performance e la salute delle vacche da latte. Diversi fattori contribuiscono allo stress da caldo tra i quali, temperatura ambientale elevata, energia radiante (luce solare diretta e riflessa), alta umidità relativa. Questi parametri possono che compromettere la capacità dell'animale di dissipare il calore corporeo e influenzare negativamente le performance produttive e riproduttive.

Quando le bovine non sono in grado di dissipare calore sufficiente a mantenere l'equilibrio termico, la loro temperatura corporea si alza e insorge lo stress da caldo. La più nota risposta allo stress da caldo è una riduzione della produzione di latte, dato facilmente misurabile. Altri parametri sono rappresentati da ridotta ingestione, ridotta fertilità e, spesso, perdita di peso corporeo. Ci sono diversi accorgimenti che si possono attuare, tra i quali la predisposizione delle strutture con idonei impianti di raffrescamento e ventilazione, management e\o modifiche nutrizionali mirate per affrontare le sfide connesse allo stress da caldo.

Meccanismi di reazione

Al fine di mantenere la temperatura corporea in condizioni di normalità, la bovina innesca una serie di "meccanismi di difesa": sudorazione, aumento della respirazione e una maggiore frequenza cardiaca. Tali meccanismi naturali possono avere ripercussioni sulle altre funzioni corporee.

Alcuni esempi:

- aumento della sudorazione:

maggior perdita di acqua e minerali, tra i quali nutrienti necessari agli organi per svolgere le loro attività metaboliche in modo efficiente. Poiché l'acqua e i sali minerali sono anche i principali componenti del latte, la produzione di latte diminuisce;

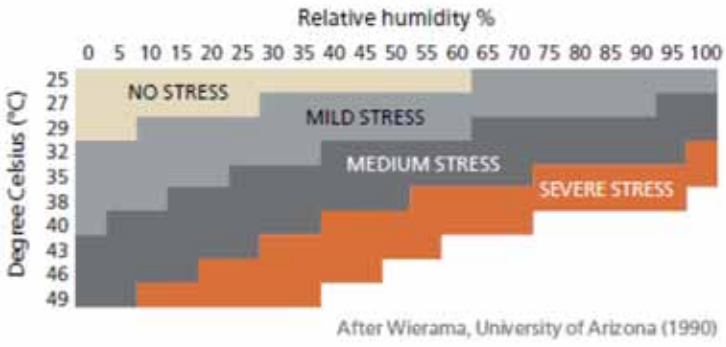
- incremento degli atti respiratori:

maggior perdita di acqua, così come anidride carbonica e bicarbonato del sangue, composti naturali tampone che, se non sufficienti, possono facilitare l'insorgenza di problematiche correlate all'acidosi e ad altri disordini metabolici.

Pratiche strategie alimentari

Minerali:

A differenza degli uomini, le vacche da latte utilizzano il potassio (K) come principale regolatore osmotico di secrezione di acqua dalle ghiandole sudoripare. Di conseguenza, i fabbisogni di potassio aumentano durante il periodo estivo.



After Wierama, University of Arizona (1990)

Lievito vivo:

considerare l'utilizzo di colture di lievito vivo che incrementino l'ingestione di sostanza secca, aumentano la digeribilità della fibra, prevengono i fenomeni di acidosi ruminale riducendo la produzione di acido lattico nel rumine.

Alltech può aiutare a sostenere la produzione e i parametri qualitativi del latte durante l'estate, supportando la funzionalità ruminale e l'ingestione di sostanza secca. Grazie all'utilizzo di tecnologie Alltech e a un livello adeguato e differenziato di minerali, siamo in grado di migliorare le performance e il benessere animale, sempre con un occhio di riguardo ai costi alimentari.

Per maggiori informazioni contatta
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UNA FINESTRA SULLE AZIENDE

zoetis

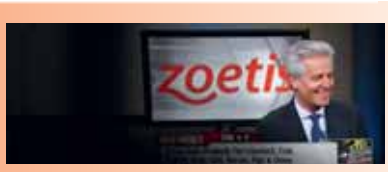
Zoetis scommette sull'Italia e punta su sviluppo, ricerca e innovazione

Sono passati tre anni da quando Zoetis è diventata un'azienda indipendente. Tre anni in cui lo scenario dell'industria della salute animale è cambiato profondamente, quella che era solo una piccola parte del panorama sanitario, con un numero limitato di investimenti, interesse e conoscenze, è diventata invece a pieno diritto un pezzo importante dell'industria della salute. E Zoetis è una delle protagoniste di questo cambiamento. Grazie a un team per la vendita diretta leader nel settore, a un comparto di Ricerca e Sviluppo produttivo, e a una produzione di altissima qualità, l'azienda è riuscita a raggiungere per tre anni consecutivi un fatturato sopra la media di mercato.

"Una parte dei nostri piani a lungo termine, è di continuare a guidare l'industria della salute animale non solo in termini di fatturato, ma puntando tutto sull'innovazione e la ricerca. È anche per questo che i nostri clienti riconoscono l'alta professionalità del nostro personale e la qualità indiscussa dei nostri prodotti. Nei prossimi anni cercheremo di essere ancora più efficienti e focalizzati, così da rimanere competitivi. Il nostro obiettivo infatti è continuare a crescere insieme ai nostri clienti per poter continuare a lavorare per la salute e il benessere animale, garantendo servizi, qualità e innovazione. Non potremmo essere così competitivi sul mercato se non avessimo alle spalle un settore di ricerca altamente qualificato", **ha dichiarato Juan Ramòn Alaix, Chief Executive Officer** della multinazionale del settore veterinario, durante la sua visita in Italia dello scorso giugno.

L'Italia, infatti, è uno dei paesi su cui Zoetis scommette: nel 2015 il fatturato in Italia è stato pari al 2% dei guadagni a livello globale, provenienti per il 55% dal settore dell'allevamento e per il 45% da quello degli animali da compagnia. "Quello italiano è attualmente fra i primi mercati mondiali per Zoetis - continua ancora Alaix - Diverse indagini ci dicono infatti che l'Italia ha la più ampia quota di mercato nel continente e non va dimenticato poi che l'Italia è importante anche per la produzione, con ben due impianti: a Catania e a Medolla".

Proprio Catania è stata una delle tappe del tour italiano di Alaix, dove ha incontrato il vice sindaco della città, Marco Consoli, il vice presidente vicario e il direttore di Confindustria etnea, Antonello Biriaco e Fabrizio Casicci. "Il sito di Catania è unico nel suo genere", ha dichiarato Alaix. "L'alto livello della produttività, la flessibilità, la peculiarità del prodotto, sono le caratteristiche che apprezziamo in questo stabilimento su cui punteremo nel prossimo futuro con maggiori investimenti".



Investire, anche per quanto riguarda la formazione dei veterinari, è la cifra distintiva di Zoetis che investe da anni nel futuro della professione, supportando la ricerca, finanziando l'educazione dei futuri veterinari e organizzando programmi di specializzazione mirati. Tra questi va senz'altro ricordata la collaborazione con l'Easter Bush Research Consortium, il più ampio consorzio europeo per la ricerca veterinaria, che punta a migliorare la prevenzione e la gestione delle patologie veterinarie. E poi la partnership con la Morris Animal Foundation, con cui da 13 anni l'azienda porta avanti il Canine Health Project, uno studio osservazionale unico, che punta a identificare fattori di rischio nutrizionali, ambientali e genetici per le malattie canine. Zoetis inoltre è partner commerciale (unico) di un'alleanza globale di università e istituti di ricerca che ha ricevuto il più ampio finanziamento europeo per un programma di ricerca sulla salute animale, assegnato nell'ambito del settimo programma quadro, per lo sviluppo di vaccini e test diagnostici per le parassitosi negli animali da allevamento. Dal 2014 inoltre Zoetis supporta l'African Small Companion Animal Network (AFSCAN), un progetto che punta a facilitare la creazione di un network sostenibile di veterinari specializzati nella salute degli animali da compagnia nel continente africano.

In Italia è attiva da anni una collaborazione tra Zoetis e la Società Italiana Veterinari per Animali da Reddito, Zoetis e Sivar hanno organizzato tra le altre cose, una serie di workshop durante il convegno nazionale, indirizzati ai giovani veterinari dal titolo Young and Dairy. Nel corso del Sivar 2016 è stato invitato l'esperto di Cow Signaling Joep Driessen, che ha tenuto un seminario su come comprendere i segnali comportamentali delle bovine da latte, per migliorarne benessere e produttività.

Per saperne di più su Zoetis, Vi invitiamo a visitare il sito www.zoetis.com

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Antibioticoresistenza nei suini organici e convenzionali

Influenzata da fattori specifici relativi sia al paese sia al sistema produttivo. Studio su 4 paesi europei, tra cui l'Italia

L'allevamento organico del suino differisce da quello convenzionale in molti aspetti, tra cui l'utilizzo di antibiotici, la struttura della mandria, il regime alimentare, l'accesso agli spazi esterni e la disponibilità di spazio per suino. Uno studio ha indagato se tali differenze determinassero una minore comparsa di antibioticoresistenza nei suini organici in Danimarca, Francia, Italia e Svezia. Si prelevavano campioni di contenuto del colon e/o di feci e si determinava la minima concentrazione inibente (MIC) di 10 antibiotici in isolati di Escherichia coli. Inoltre, si determinava la percentuale di E. coli resistenti alla tetraciclina (TET) nel contenuto del colon e/o nelle feci di singoli suini.

In tutti i quattro i paesi, la percentuale di resistenza ad ampicillina, streptomicina, sulfonamidi o trimetoprim era significativamente inferiore in E. coli dei suini organici. In Francia e in Italia, anche la percentuale di isolati resistenti a cloramfenicolo, ciprofloxacina, acido nalidixico o gentamicina era significativamente inferiore in E. coli dei suini organici. In nessuno dei paesi si riscontrava resistenza a cefotaxime.

La percentuale di isolati di E. coli resistenti a TET così come la percentuale di E. coli TET-resistenti era significativamente inferiore nei suini organici rispetto a quelli convenzionali, fatta eccezione per la Svezia in cui la resistenza a TET era ugualmente bassa in entrambi i sistemi produttivi.

Si osservavano inoltre differenze tra i paesi all'interno dei sistemi produttivi nella percentuale di resistenza ai singoli antibiotici così come nella percentuale di E. coli TET-resistenti, con percentuali mediane minori in Svezia e Danimarca rispetto a Francia e Italia.

Lo studio indica che, in ciascuno dei quattro paesi analizzati, la resistenza di E. coli intestinale era meno frequente nei suini organici rispetto a quelli convenzionali, ma si osservavano anche evidenti differenze di resistenza tra i paesi all'interno di ciascun sistema produttivo, indicando che fattori specifici sia relativi al paese sia alla produzione influenzano la comparsa di resistenza, concludono gli autori.

"Antibiotic Resistance in Escherichia coli from Pigs in Organic and Conventional Farming in Four European Countries." Österberg J, et al. PLoS One. 2016 Jun 30; 11(6).

Previsione delle infezioni intramammarie durante l'asciutta

La durata dell'asciutta non influenzava la probabilità di nuove IMI, in uno studio

Il periodo dell'asciutta è molto importante per la salute della mammella, non solo al fine della cura delle infezioni intramammarie (IMI) esistenti ma anche per prevenire nuove IMI. Tuttavia, la probabilità che singole bovine sviluppino nuove IMI o, se infette, non vengano curate, non è ben stabilita.

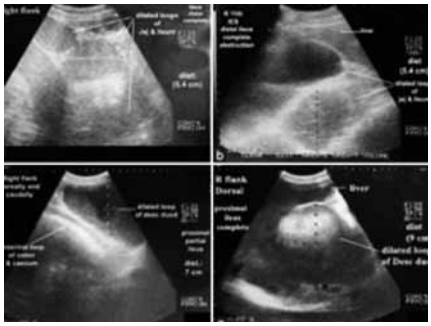
Uno studio ha indagato se i dati relativi alle bovine per tutta la durata della vita, disponibili grazie a registrazioni routinarie delle lattazioni in allevamento, potessero essere utilizzati per prevedere i cambiamenti nello status IMI durante il periodo dell'asciutta per le singole bovine con 1) elevata conta delle cellule somatiche (SCC; >199.000 cell/mL) o (2) bassa SCC (<200.000 cell/mL) all'ultimo giorno di test prima dell'asciutta.

Si raccoglievano i dati relativi alle lattazioni in 114 mandrie. Le bovine con una minore proporzione di giorni di test con SCC elevata durante la lattazione prima dell'asciutta, una minore proporzione di giorni di test con SCC elevata nella lattazione precedente la lattazione attuale, con ordine di parto minore, che producevano meno latte prima dell'asciutta, con minori giorni d lattazione all'asciutta e una minore SCC subito prima dell'asciutta avevano una maggiore probabilità di essere curate durante l'asciutta. La durata dell'asciutta non aveva effetti sulla probabilità di cura.

Le bovine con una minore proporzione di giorni di test con SCC elevata durante la lattazione precedente quella attuale, minore ordine di parto, minore produzione di latte all'asciutta e minore numero di giorni di lattazione all'asciutta avevano una minore probabilità di sviluppare una nuova IMI.

Si riscontrava che la durata dell'asciutta non aveva effetti sulla probabilità di nuove IMI. I modelli predittivi mostravano che era possibile un elevato grado di discriminazione tra bovine con rischio elevato e basso sia di cura sia di nuove infezioni durante l'asciutta, concludono gli autori.

"Prediction of intramammary infection status across the dry period from lifetime cow records" A.C. Henderson et al. Journal of Dairy Science. July 2016. Volume 99, Issue 7, Pages 5586-5595.



Ostruzione intestinale nel bufalo: esame ecografico

Uno strumento essenziale per la diagnosi e la differenziazione tra diverse forme ostruttive

In uno studio è stata effettuato una valutazione clinica e di laboratorio delle ostruzioni intestinali nel bufalo (Bubalus bubalis), con particolare riguardo al valore diagnostico dell'esame ecografico.

Si includevano in totale 30 bufali suddivisi in 2 gruppi: sani (n=10) e ammalati (n=20).

I soggetti ammalati avevano un'anamnesi di anoressia, dolore addominale, gradi variabili di distensione addominale e assenza o presenza di scarse feci mucoidi.

Sulla base dell'esame ecografico, si diagnosticavano diverse forme di ostruzione intestinale. L'ostruzione funzionale, o ileo paralitico, veniva diagnosticata in 17 casi (85%), mentre l'ostruzione meccanica veniva osservata solo in 3 casi (15%).

Nei 17 casi di ileo paralitico, si potevano evidenziare efficacemente l'ileo prossimale e distale rispettivamente in 8 e 9 casi. L'ileo prossimale veniva visualizzato dalla regione dorsale del fianco destro come una singola ansa dilatata di diametro > 6 cm, mentre l'ileo distale veniva visualizzato come anse dilatate multiple di diametro < 6 cm. L'ostruzione meccanica dovuta a intussuscezione duodenale veniva visualizzata in forma di due due anelli concentrici con una parete esterna ecogena e un lume ipoecogeno.

Tutti i casi di ostruzione intestinale presentavano anche leucocitosi, ipoproteinemia e aumento dell'attività della fosfatasi alcalina e dell'aspartato aminotransferasi.

L'esame ecografico si dimostrava uno strumento essenziale per la diagnosi e la diagnosi differenziale dell'ostruzione intestinale nel bufalo, concludono gli autori.

"Clinical and ultrasonographic observations of functional and mechanical intestinal obstruction in buffaloes (Bubalus bubalis)." Khalphallah A, Aref NE, Elmeligy E, El-Hawari SF. Vet World. 2016 May; 9 (5): 475-80.

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