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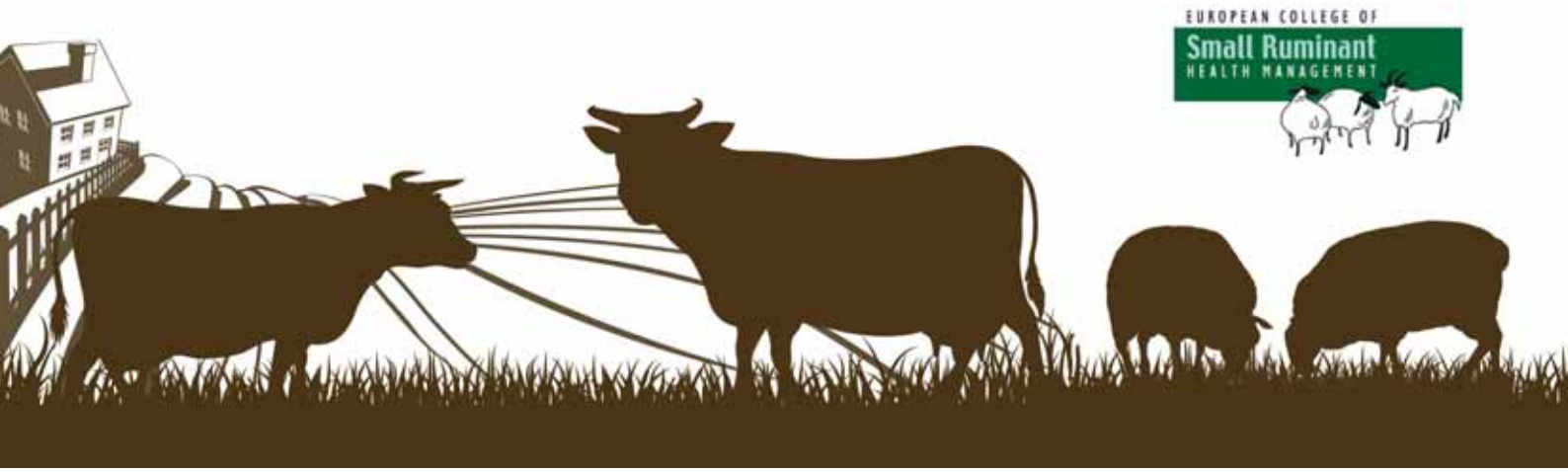

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Consumo di antimicrobici, benessere animale e biosicurezza in 16 aziende di bovine da latte in Lombardia

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RIASSUNTO

Il benessere degli animali, il consumo di antimicrobici e la biosicurezza sono le nuove sfide che l'allevamento della bovina da latte deve affrontare. Tre aspetti sempre più connessi tra di loro, la cui gestione richiede un approccio integrato. Questo studio ha voluto testare, per la prima volta sul campo, il nuovo sistema integrato ClassyFarm in 16 aziende di bovine da latte situate in Lombardia. Esso consente la categorizzazione degli allevamenti in base rischio in relazione al loro livello di benessere animale, di biosicurezza e all'impiego degli antimicrobici.

Nei 16 allevamenti oggetto di studio, i livelli di benessere e biosicurezza sono stati determinati utilizzando il protocollo di valutazione messo a punto dal Centro di Referenza Nazionale per il Benessere Animale (CReNBA) ed inserito nel sistema ClassyFarm. Il consumo di antimicrobici (AMU) delle bovine adulte, espresso come giorni standard di trattamento a cui una vacca è stata potenzialmente esposta nel corso del periodo di riferimento ("giorni/capo per anno"), è stato misurato utilizzando le "Defined Daily Dose Animal for Italy" (DDDAit).

Il punteggio di benessere animale, riferito alle bovine adulte, è risultato in media pari a 67,98% (*range* 45,32% - 81,69%), il punteggio medio di biosicurezza è stato del 45,86% (*range* 21,41% - 71,56%); inoltre il sistema ha consentito di identificare i principali punti critici in questi due ambiti, come ad esempio l'igiene e la gestione non adeguate dello spazio adibito al preparto/parto, la mancanza di una struttura specifica adibita ad infermeria oppure la mancanza di una distanza adeguata tra automezzi in entrata e le aree di stabulazione degli animali.

Negli allevamenti indagati, la media dell'AMU è risultata pari a 10,57 giorni/capo per anno (*range* 5,13 - 19,61). Gli antimicrobici nelle bovine adulte sono stati principalmente utilizzati per le patologie mammarie (42,03%), per la terapia profilattica delle bovine in asciutta (35,77%), per le patologie locomotorie (12,74%), urogenitali (5,79%) e respiratorie (2,36%). Le penicilline, le cefalosporine di terza e quarta generazione sono state le classi di antimicrobici maggiormente impiegate. Le vie di somministrazione più utilizzate sono state la via iniettabile (43,57%) e la via intramammaria (34,67% per le bovine in asciutta e 20,34% per le bovine in lattazione). Non sono state identificate correlazioni statisticamente significative tra l'AMU e le altre variabili studiate (livello di benessere animale e livello di biosicurezza); tuttavia, data la scarsa numerosità del campione, sono necessarie ulteriori indagini per approfondire i risultati ottenuti.

Il sistema integrato ClassyFarm si è rivelato uno strumento molto promettente per le aziende di bovine da latte italiane per monitorare e migliorare i livelli di benessere animale e di biosicurezza, individuando i punti critici sui quali intervenire, e per promuovere un utilizzo più razionale dell'antimicrobico.

PAROLE CHIAVE

Benessere delle vacche da latte; valutazione del benessere; uso antimicrobico; dose giornaliera definita.

INTRODUZIONE

L'uso frequente e spesso improprio degli antimicrobici ha favorito la selezione e la diffusione di microrganismi multi-resistenti che, in questi anni, ha suscitato una forte attenzione nelle istituzioni nazionali ed internazionali, in quanto responsabile di fallimenti terapeutici, di maggiori tassi di ospedalizzazione e di un maggior numero di decessi, diventando uno dei principali problemi di sanità pubblica[1]. Data la stretta connessione fra salute umana, animale ed ambiente, l'antimicrobicoresistenza (AMR) deve essere affrontata mediante un approccio *One Health.* Numerose ricerche hanno evidenziato una relazione fra l'utilizzo di antimicrobici in ambito zootecnico e lo sviluppo dell'AMR, anche nei confronti di medicinali ad uso umano, sebbene sia difficile valutarne con precisione l'impatto quantitativo sulla salute pubblica[2].

Nell'allevamento della bovina da latte l'impiego degli antimicrobici è generalmente rivolto alla terapia delle mastiti cliniche e subcliniche, delle polmoniti, delle metriti, delle infezioni podali e al trattamento profilattico al momento dell'asciutta[3]. Tuttavia, se da un lato l'utilizzo degli antimicrobici è un'azione imprescindibile che ha come obiettivo la cura degli stati patologici, la riduzione delle sofferenze e il mantenimento di un buono stato di benessere animale[4], dall'altro il loro utilizzo eccessivo o inappropriato può determinare lo sviluppo di resistenze, che provocano il fallimento delle tera-

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pie e quindi il perdurare dello stato di malattia e di malessere degli animali.
Un adeguato livello di benessere animale in allevamento deve essere raggiunto e mantenuto non attraverso l'utilizzo indiscriminato dei trattamenti antimicrobici, ma mediante una riduzione dell'incidenza delle malattie e permettendo all'animale di massimizzare le proprie capacità di adattamento all'ambiente, riducendo i fattori stressanti per mezzo di efficaci programmi di gestione aziendale, di profilassi, di biosicurezza e di potenziamento delle strutture di allevamento[5].
La Direzione Generale della Sanità Animale e dei Farmaci Veterinari (DGSAF) del Ministero della Salute ha finanziato un progetto multidisciplinare con l'obiettivo di individuare indicatori utili per la categorizzazione delle aziende zootecniche in base al livello di rischio sanitario, di benessere animale e di consumo di antimicrobici, al fine di ridurre lo sviluppo dell'AMR[6]. Il piano di lavoro ha portato alla messa a punto del sistema di monitoraggio integrato "ClassyFarm" (www.classyfarm.it). All'interno della piattaforma ClassyFarm, inserita nel "Portale dei Sistemi Informativi Veterinari" (www.vetinfo.it) sono immesse, in un sistema di afflusso costante, grazie al contributo dei veterinari aziendali, informazioni riguardanti diversi ambiti della sanità animale: benessere animale, biosicurezza, parametri sanitari e produttivi, alimentazione, consumo di farmaci antimicrobici e rilievo delle lesioni al macello. Dalla loro raccolta ed elaborazione sono prodotti degli indicatori numerici, scientificamente validati, indicanti il livello di rischio dell'allevamento.
In ClassyFarm, le informazioni sul consumo di antimicrobici sono elaborate mediante l'utilizzo della DDDAit (*Defined Daily Dose Animal for Italy*), unità di misura standardizzata per la medicina veterinaria basata sulle posologie degli antimicrobici disponibili in Italia e in grado di fornire una stima della potenziale esposizione della mandria agli antimicrobici. L'approccio è simile a quello della *"defined daily dose for animals"* (DDDvet) messa a disposizione dall'ESVAC e risultato di un'ampia analisi sulle posologie di tutti gli antimicrobici disponibili in nove Paesi europei[7], tra i quali però non era presente l'Italia. L'applicazione di metriche basate sulla DDD in medicina umana è iniziata oltre mezzo secolo fa e, già dal 1981, l'Organizzazione Mondiale della Sanità (OMS) raccomandava la DDD come standard internazionale per gli studi e le analisi sull'utilizzo dei farmaci[8]. Le azioni volte alla riduzione dell'uso di antimicrobici possono, infatti, essere realizzate solo con un efficace sistema di controllo, in grado di misurare in modo appropriato il consumo degli stessi.
Questo studio preliminare vuole mettere in evidenza le potenzialità del sistema integrato ClassyFarm, raccogliendo informazioni sul consumo di antimicrobici (AMU), la biosicurezza ed il benessere animale ed indagando le possibili relazioni tra questi tre aspetti dell'allevamento in 16 aziende di bovine da latte in Lombardia.

MATERIALI E METODI

Selezione degli allevamenti

Attraverso un campionamento di convenienza, sono stati selezionati 16 allevamenti di bovine da latte a stabulazione libera situati in Regione Lombardia. Gli allevamenti appartenevano ad un gruppo di aziende (n=42) che nel corso del 2015 avevano partecipato a progetti di ricerca del Centro di Referenza Nazionale per il Benessere Animale (CReNBA). I 16 allevamenti soddisfacevano i criteri di inclusione stabiliti per questo studio: allevamenti a stabulazione libera, numero di bovine adulte > 30, localizzazione in Regione Lombardia e disponibilità dell'allevatore a permettere la completa visualizzazione del registro dei trattamenti.
Durante il triennio 2016-2018, nei 16 allevamenti selezionati è stato applicato il protocollo messo a punto dal CReNBA per la Valutazione del Rischio applicata al Benessere Animale e alla Biosicurezza e sono stati raccolti tutti i dati necessari per il calcolo del consumo di antimicrobici. Durante la visita in azienda, in tutti gli allevamenti sono stati raccolti, inoltre, i dati riportati in Tabella 1: numero medio di capi in lattazione e in asciutta, numero medio di manze (femmine con età > 6 mesi fino al giorno prima del parto), numero medio di vitelli (maschi e femmine ≤ 6 mesi di età), quantità di latte consegnato in un anno espressa in quintali, dalla quale è stata ottenuta la media aritmetica della produzione giornaliera per capo (kg/capo/giorno), e il tasso di mortalità per le tre classi di animali (bovine adulte, manze e vitelli), calcolato prendendo in considerazione i capi trovati morti spontaneamente, eutanasizzati o macellati d'urgenza.

Valutazione del Rischio applicata al Benessere Animale e alla Biosicurezza in allevamento

Nei 16 allevamenti selezionati è stato valutato il livello di benessere animale e di biosicurezza, utilizzando il protocollo CReNBA, incluso nel sistema ClassyFarm e composto da una check-list (denominata "BOVINA LATTE LIBERA - Benessere" e reperibile sul sito www.classyfarm.it) a risposta multipla con 85 osservazioni, ognuna delle quali ha un peso diverso in funzione del possibile impatto sul benessere animale o sulla sanità degli animali[9]. Due allevamenti sono stati valutati a fine 2016 e 14 allevamenti durante il 2017.
Per la valutazione del livello di benessere animale, il metodo si basa sull'analisi di due gruppi di dati: 1) indicatori *non-animal based* (N-ABM), legati da un lato alla gestione dell'azienda e alla formazione del personale (inclusi nell'Area A - "Management aziendale e personale", 23 item) e dall'altro alle condizioni strutturali dell'allevamento (Area B - "Strutture ed attrezzature", 29 item); 2) indicatori *animal-based* (ABM), in grado di fornire una valutazione diretta delle condizioni degli animali (Area C - "Animal-based measures", 18 item). Il risultato parziale di ogni area è calcolato sommando i risultati ottenuti dall'allevamento per ciascun indicatore N-ABM (x) e ABM (y). Il valore complessivo del benessere animale (PBA), misurato su una scala da 0 a 100% (dove 0% indica il punteggio peggiore e 100% il punteggio migliore), è il risultato del contributo pari al 50% per entrambe le categorie di indicatori ed è calcolato con la seguente equazione:

$$PBA = 0{,}5 \sum_i x_i + 0{,}5 \sum_i y_i$$

Dove:
x_i indica il valore dell'i-esima misura *non-animal based* (52 misure);
y_i indica il valore dell'i-esima misura *animal-based* (18 misure).

Per la valutazione del livello di biosicurezza, la check list applicata ("BOVINA LATTE LIBERA - Benessere") comprendeva 15 osservazioni (Area Biosicurezza) mirate all'individuazione dei maggiori rischi sanitari in allevamento. Come per la valutazione del livello di benessere animale, anche per la valutazione del livello di biosicurezza, ogni condizione è

Tabella 1 - Dimensione della mandria, mortalità annuale e dati produttivi delle 16 aziende oggetto di studio. Le aziende sono ordinate in modo crescente in base al punteggio totale di benessere animale ricalcolato per le bovine adulte (PBA_{adulte}).

Azienda	Capi presenti in allevamento mediamente in un anno					Mortalità annuale*			Latte consegnato			
	Totale	Lattazione	Asciutta	Manze	Vitelli	% Bovine adulte	% Manze	% Vitelli	Quintali/ anno	Latte capo giorno (kg)	MGBTSCC** (cell/ml)	MGCBT** (ufc/ml)
1	79	29	3	16	31	0	0	0	2167	20,47	247225	28206
2	140	40	10	40	50	14	2,5	14	3500	23,97	243060	18369
3	615	184	27	187	217	4,74	0	3,23	18700	27,84	192101	23407
4	464	140	17	145	162	5,1	0,69	5,56	15500	30,33	268484	17647
5	151	47	8	45	51	3,6	4,4	7,8	4400	25,65	323401	12675
6	300	107	10	50	133	25,64	0	26,32	12869	32,95	203421	28305
7	272	97	20	58	97	5,13	1,72	10,31	11403	32,21	180435	11377
8	515	160	50	160	145	2,86	1,88	30,35	19200	32,88	162447	13865
9	244	95	17	65	67	3,6	0	3	9293	26,8	215838	7400
10	340	105	20	75	140	3,2	1,33	10,71	10585	27,62	206566	25521
11	617	180	38	214	185	2,29	0	7,57	18846	28,69	222294	17852
12	200	80	15	31	74	2,1	0	0	9000	30,82	273228	6636
13	354	160	30	40	124	5,26	0	3,23	14300	24,49	283646	15161
14	266	96	14	75	81	0	0	2,47	12716	36,29	152322	8214
15	524	165	18	150	191	0,55	0	2,09	21000	34,87	143904	4436
16	171	52	10	47	62	3,2	0	6,5	2001	10,54	203421	28305
Media	328	109	19	87	113	5,08	0,78	8,32	11592,5	27,90	220112	16711
Minimo	79	29	3	16	31	0,00	0,00	0,00	2001	10,54	143904	4436
Massimo	617	184	50	214	217	25,64	4,40	30,35	21000	36,29	323401	28305

*Mortalità annuale: sono compresi gli animali morti spontaneamente, eutanasizzati e le macellazioni speciali di urgenza.
**MGBTSCC: media aritmetica di un anno del parametro Media Geometrica delle Cellule Somatiche del latte di massa; MGTBC: media aritmetica di un anno del parametro Media Geometrica delle Carica Batterica Totale.

associata ad un peso, espressione del suo possibile impatto sulla sanità degli animali. L'indice di biosicurezza è calcolato come sommatoria dei pesi dei singoli indicatori ed è espresso su una scala da 0 a 100% (con l'aumentare del punteggio si hanno migliori condizioni di biosicurezza).

Estrazione dei dati relativi ai parametri del latte di massa

Dal database del Centro di Referenza Nazionale per la Qualità del Latte Bovino sono state estratte, per ciascuna delle 16 aziende, i risultati delle analisi sul latte di massa eseguite nell'anno della valutazione del benessere animale (2016 o 2017) e relative a: media geometrica delle cellule somatiche (MGBTSCC) e media geometrica della carica batterica totale (MGBTCBT) e ne è stata calcolata la media aritmetica per l'anno di riferimento.

Valutazione del consumo di antimicrobici mediante il sistema ClassyFarm

Durante il biennio 2017-2018, nei 16 allevamenti selezionati sono stati raccolti retrospettivamente i dati per il calcolo dell'AMU relativi all'anno in cui è stata effettuata la valutazione del benessere animale (2016 o 2017). In particolare, è stata eseguita una scansione del registro dei trattamenti e i dati raccolti sono stati inseriti all'interno di un database XML, così da calcolare la quantità di ciascuna specialità medicinale utilizzata nelle bovine adulte e il motivo del trattamento (patologie respiratorie, cutanee, nervose, urogenitali, locomotorie, enteriche, setticemiche, mammarie, osteoarticolari, oculari, profilassi vaccinale, trattamenti per asciutta). Infine si è proceduto al calcolo delle DDDAit, per singolo principio attivo.

In linea generale, una DDDAit rappresenta la dose, in milligrammi, di principio attivo che dovrebbe essere somministrata per tenere sotto trattamento un chilogrammo di peso vivo nell'arco di ventiquattro ore, secondo le indicazioni definite dal "Riassunto sulle Caratteristiche del Prodotto" di ogni farmaco antimicrobico. Fanno eccezione a tale definizione le DDDAit dei prodotti intramammari ed intrauterini: tali prodotti non prevedono un dosaggio basato sul peso vivo e, pertanto, la loro dose è stata misurata in modo unitario (es. numero di tubi-siringa da somministrare al giorno). Le esposizioni a tutti i principi attivi somministrati durante il periodo di riferimento sono state calcolate singolarmente, anche se tali molecole facevano parte di un'associazione.

Al fine del presente studio, non è stato possibile impiegare direttamente le DDDvet descritte da ESVAC[7] poiché esse, allo stato attuale, risultano ancora incomplete. Nella fattispecie, non sono disponibili le DDDvet per: antimicrobici ad uso intramammario somministrabili durante l'asciutta; alcuni macrolidi *long acting* (tildipirosina e gamitromicina); alcuni farmaci registrati in Italia (es. dicloxacillina iniettabile).

Le DDDAit consumate nel periodo di riferimento sono state rapportate alla biomassa (numero di animali per il peso standard della categoria, che per le bovine adulte è stato fissato a 600 kg) degli animali presenti in allevamento durante il medesimo arco temporale. Tale rapporto (AMU) esprime il numero di giorni di trattamento cui ciascun soggetto presente in azienda è stato potenzialmente esposto durante il periodo di riferimento ed è stato ottenuto attraverso la seguente formula [modificata da Timmerman e collaboratori (2006)][10].

$$AMU\ (\frac{giorni}{capo}\ per\ anno) = \frac{DDDAit\ consumate\ nel\ periodo\ di\ riferimento\ (2016\ o\ 2017)}{Presenza\ media\ di\ vacche\ nel\ periodo\ x\ Peso\ Standard}$$

Per gli antimicrobici ad uso intrauterino (IU) ed intramammario (IM), l'AMU è stato calcolato non includendo nella formula il peso standard al trattamento, vale a dire:

$$AMU_{IM\ e\ IU}\ (\frac{giorni}{capo}\ per\ anno) = \frac{DDDAit\ consumate\ nel\ periodo\ di\ riferimento\ (2016\ o\ 2017)}{Presenza\ media\ di\ vacche\ nel\ periodo}$$

Nel caso degli antimicrobici IM per la lattazione è stato considerato lo standard di due mungiture al giorno, mentre per gli antimicrobici IM per l'asciutta non è stato possibile determinare la dose giornaliera, pertanto si è fatto rifermento a quanto descritto in letteratura[11].
Il sistema ClassyFarm, nella sua componente relativa al consumo di antimicrobici, non fornisce un singolo dato aggregato per azienda, bensì dei valori di consumo suddivisi per categoria zootecnica (es. vitello, manza, bovina adulta). In questo studio sono stati utilizzati solo i valori relativi alla categoria "bovina adulta".

Analisi dei dati

Ai fini di questo lavoro preliminare, il PBA e i punteggi parziali di area A, B e C, ottenuti dall'applicazione del protocollo di valutazione del benessere animale, sono stati ricalcolati sottraendo gli item riferiti al benessere delle manze e dei vitelli, in modo da considerare solo il benessere delle bovine adulte (lattazione e asciutta). In tal modo è stato possibile porli in relazione con l'AMU di tale categoria produttiva.
Dopo aver effettuato un'analisi descrittiva preliminare di ogni azienda, è stata osservata la relazione tra PBA_{adulte} e l'AMU e fra il Punteggio Biosicurezza e l'AMU. Le aziende sono state poi suddivise in quartili (Q1-Q4) in base al punteggio PBA_{adulte} e denominate, per praticità, a "benessere scarso" (Q1), "benessere sufficiente" (Q2), "benessere buono" (Q3), "benessere ottimo" (Q4) e confrontate con il relativo AMU.
Il PBA_{adulte} e i punteggi parziali di area (Area A_{adulte}, Area B_{adulte}, Area C_{adulte}) di ciascuna azienda, il Punteggio di Biosicurezza, i parametri MGBTSCC e MGCBT sono stati correlati con il rispettivo AMU. Vista la natura preliminare dello studio e dato il numero limitato degli allevamenti coinvolti e la distribuzione non normale dei dati, è stato deciso di utilizzare una misura statistica non parametrica di correlazione al fine di ottenere delle prime indicazioni sull'esistenza di possibili relazioni tra i parametri indagati. Per questo motivo è stato utilizzato il coefficiente di correlazione per ranghi di Spearman per calcolare la relazione tra i suddetti parametri.

RISULTATI

La dimensione della mandria, la mortalità annuale degli animali e i dati produttivi delle 16 aziende oggetto di studio sono riportati in Tabella 1.
La media del PBA_{adulte} è stata pari a 67,98% (*range* 45,32% - 81,69%), la media del punteggio di Area A_{adulte} è stata pari a 67,93% (*range* 40,17% - 87,20%), la media dell'Area B_{adulte} è stata di 64,50% (*range* 30,88% - 80,84%), la media del punteggio di Area C_{adulte} è stata pari a 69,74% (*range* 55,12% - 85,04%) e la media del Punteggio di Biosicurezza è stata di 45,86% (*range* 21,41% - 71,56%). La media dell'AMU nelle bovine adulte è stata pari a 10,57 giorni/capo per anno (*range* 5,13 - 19,61) (Tabella 2).

Tabella 2 - Risultati ottenuti nei 16 allevamenti di bovine da latte relativi a: punteggio totale benessere animale (PBA_{adulte}, scala da 0 a 100%), punteggio di biosicurezza (scala da 0 a 100%) e consumo di antimicrobici nelle bovine adulte (AMU). Nello specifico, per l'AMU sono riportati i dati totali e i dati per i prodotti intramammari in lattazione (IM LAT) o in asciutta (IM ASC) e per la terapia della mastite. Le aziende sono ordinate in modo crescente in base al PBA_{adulte}.

Azienda	Benessere Animale (%) e Biosicurezza (%)					Consumo di antimicrobico - AMU (giorni/capo per anno)			
	PBA_{adulte}	Area A	Area B	Area C	Biosicurezza	Totale	IM LAT	IM ASC	Mastite*
1	45,32	40,17	30,88	55,12	36,48	18,37	2,25	9,38	15,68
2	51,06	46,25	45,54	56,20	21,41	16,65	3,75	2,36	10,83
3	60,64	56,71	61,75	62,01	56,24	6,47	1,21	3,12	5,38
4	64,01	65,11	57,49	66,73	58,77	6,82	1,58	2,52	5,69
5	65,82	61,36	63,08	69,39	45,40	7,59	2,42	1,31	5,83
6	66,55	75,40	76,58	57,09	46,13	13,95	1,24	9,64	11,26
7	68,20	65,79	68,37	69,29	49,12	5,21	0,77	2,26	3,63
8	69,82	70,33	69,38	69,78	43,36	7,08	3,01	2,09	5,27
9	70,43	74,03	70,68	68,50	38,61	7,08	0,23	5,57	6,50
10	70,43	63,47	70,73	73,72	71,56	8,17	2,69	2,72	5,89
11	70,58	74,50	61,44	73,23	29,26	19,61	5,34	6,74	15,30
12	71,47	77,89	63,60	72,24	25,79	5,13	1,13	1,58	2,91
13	71,97	74,44	65,83	73,82	31,85	12,37	0,00	2,68	11,71
14	79,76	68,00	80,84	85,04	53,26	9,99	2,62	2,29	7,21
15	79,93	87,20	73,76	79,43	63,37	15,62	3,29	6,59	13,79
16	81,69	86,27	72,07	84,25	63,19	8,95	0,41	2,52	3,25
Media	67,98	67,93	64,50	69,74	45,86	10,57	2,00	3,96	8,13
Minimo	45,32	40,17	30,88	55,12	21,41	5,13	0	1,31	2,91
Massimo	81,69	87,20	80,84	85,04	71,56	19,61	5,34	9,64	15,68

*Sono compresi i trattamenti antimicrobici somministrati per via iniettabile e i trattamenti in asciutta.

La distribuzione dell'AMU rispetto al PBA_{adulte} e al Punteggio di Biosicurezza è rappresentata in Figura 1 e 2.

Per quanto riguarda i trattamenti antimicrobici, le vie di somministrazione più utilizzate sono state la via iniettabile (43,6%), seguita dalla via intramammaria per le bovine in asciutta (34,7%) e in lattazione (20,3%) ed infine dagli antimicrobici ad uso intrauterino (1,4%). In Figura 3 si possono osservare le classi antimicrobiche più utilizzate espresse in "giorni/capo per anno" (AMU), evidenziate in rosso quelle considerate criticamente importanti e ad alta priorità (*Highest priority Critically Important antimicrobials* - HPCIA) secondo le Linee Guida OMS[12].

In Tabella 3 sono invece indicate le classi antimicrobiche più utilizzate, distinte in base al motivo del trattamento e in Figura 4 è rappresentato graficamente il confronto tra il consumo di HPCIA e non-HPCIA, per ogni motivo di trattamento. Le patologie mammarie e i trattamenti in asciutta (rispettivamente il 42,03% e il 35,77% di tutti i giorni di trattamento consumati dalle aziende) hanno rappresentato i motivi principali per cui sono stati usati gli antimicrobici, seguite dalle patologie locomotorie (12,74%), urogenitali (5,79%) e respiratorie (2,36%) (Figura 5).

In Figura 6, è riportato il confronto tra l'AMU e il PBA_{adulte} per ogni categoria di benessere (scarso, sufficiente, buono e ottimo).

L'elaborazione statistica (coefficiente di correlazione per ranghi di Spearman) non ha evidenziato, in nessun caso, correlazioni significative tra i diversi parametri indagati (PBA_{adulte}, Area A_{adulte}, Area B_{adulte}, Area C_{adulte}, Punteggio di Biosicurezza, MGBTSCC, MGCBT e AMU).

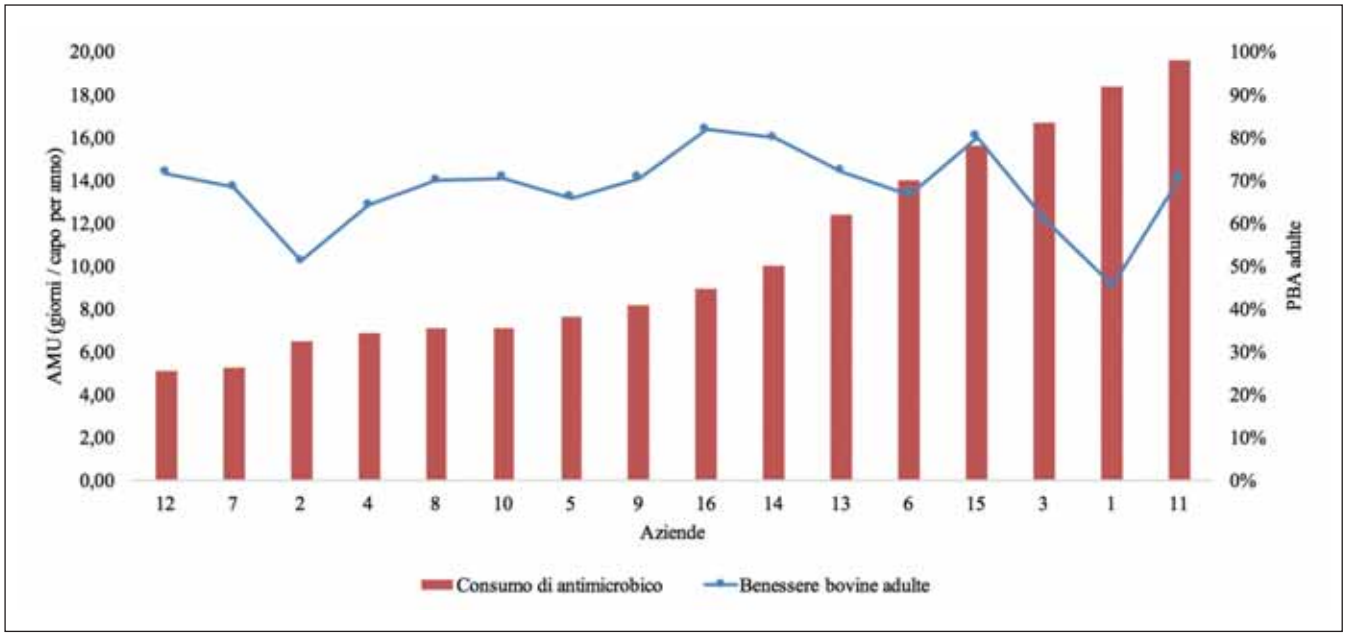

Figura 1 - Distribuzione del consumo di antimicrobici (AMU) nelle bovine adulte, espresso in giorni/capo per anno, e del Punteggio di Benessere Animale (PBA_{adulte}) nelle 16 aziende oggetto di studio.

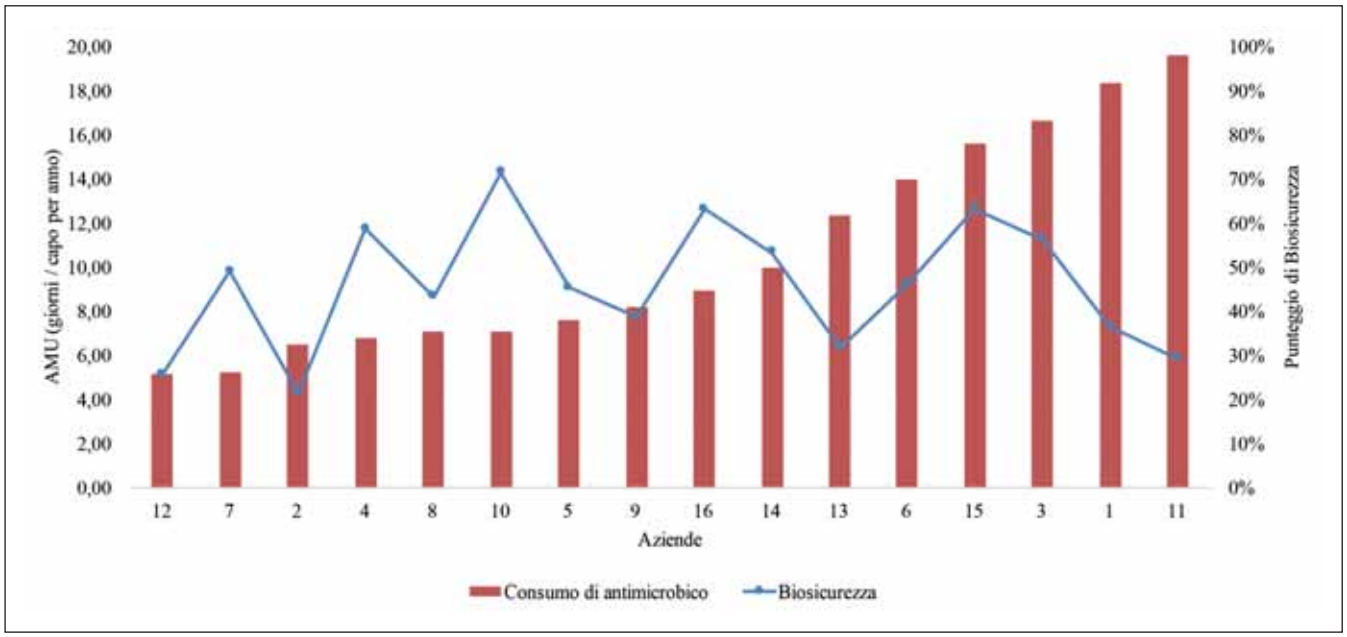

Figura 2 - Distribuzione del consumo di antimicrobici (AMU) nelle bovine adulte, espresso in giorni/capo per anno, e del Punteggio di Biosicurezza nelle 16 aziende oggetto di studio.

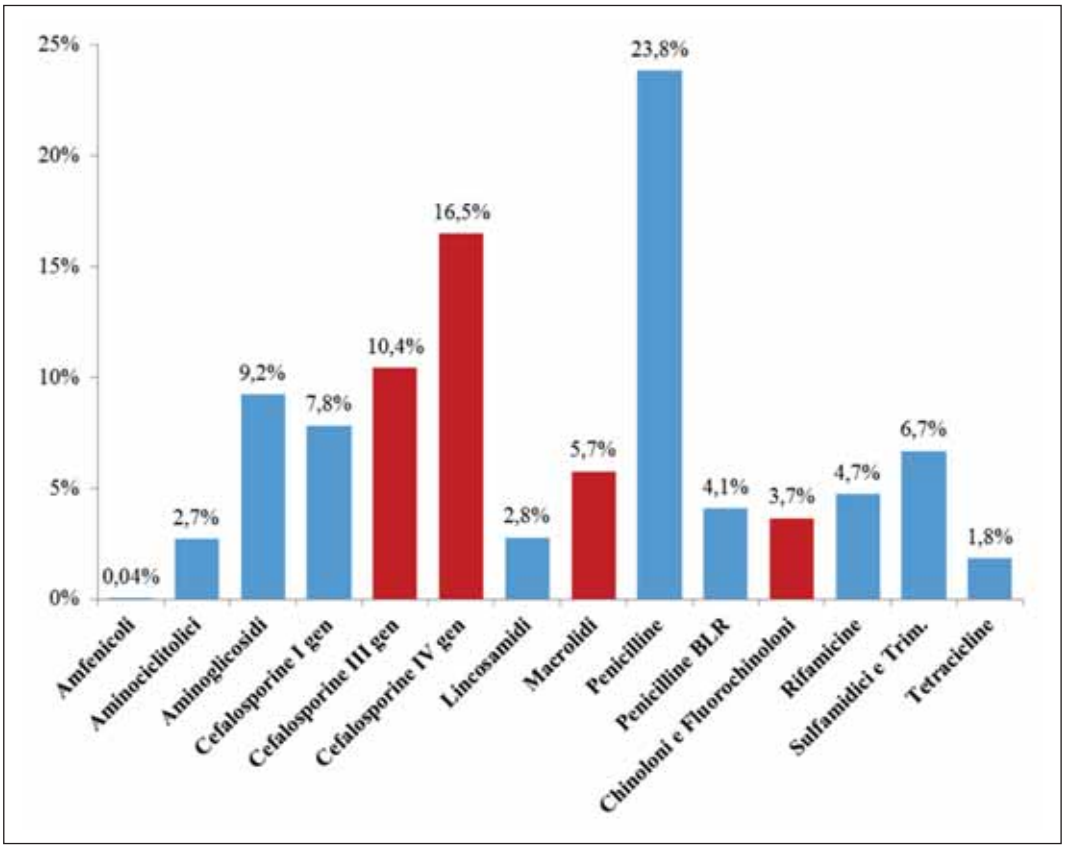

Figura 3
Classi di antimicrobico più utilizzate, calcolate sulla media ponderata dei giorni/capo per anno (AMU), nelle 16 aziende oggetto di studio. In rosso sono evidenziate le classi di importanza critica ad alta priorità (*Highest priority Critically Important antimicrobials* - HPCIA), secondo la classificazione OMS[12].

DISCUSSIONE

Confrontando i risultati relativi alla valutazione del benessere animale prodotti da questo studio con quelli ottenuti dal CReNBA, nel periodo 01/01/2011-17/11/2013 in 557 allevamenti di bovine da latte, l'Area A è risultata mediamente più scarsa (rispettivamente 67,93% e 75,20%), mentre l'Area B e C sono risultate quasi sovrapponibili (rispettivamente 64,50% e 69,74% in questo studio e 63,78% e 69,88% nello studio precedente)[13].

Per quanto riguarda il management aziendale (Area A), i punti critici più frequentemente riscontrati nelle 16 stalle oggetto di studio sono stati: la gestione dello spazio adibito al pre-parto/parto (8/16 aziende), l'igiene e la gestione dello spazio adibito al decubito delle bovine in asciutta (4/16) e la pulizia dei pavimenti e delle aree di camminamento delle bovine in lattazione (4/16). L'Area B (strutture ed attrezzature) ha presentato maggiori criticità relativamente all'assenza di una struttura specifica adibita ad infermeria (7/16), un insufficiente numero di abbeveratoi

Tabella 3 - Classi di antimicrobico più utilizzate [classificazione OMS[12]] suddivise per motivo di trattamento nelle 16 aziende oggetto di studio, espresse in percentuale di giorni/capo per anno (AMU).

Classe antimicrobica	Patologie mammarie	Trattamento in asciutta	Patologie locomotorie	Patologie urogenitali	Patologie respiratorie
Cefalosporine IV gen	13,40%	29,67%	1,20%	1,40%	0,00%
Macrolidi	11,44%	1,83%	0,11%	0,47%	5,55%
Chinoloni e Fluorochinoloni	7,61%	0,00%	0,00%	0,00%	12,96%
Cefalosporine III gen	5,04%	0,00%	64,47%	1,25%	0,69%
Penicilline	14,77%	38,94%	6,44%	40,42%	10,02%
Cefalosporine I gen	12,90%	6,69%	0,00%	0,00%	0,00%
Sulfamidici e Trim.	10,25%	0,68%	7,26%	2,52%	28,17%
Rifamicine	9,87%	0,15%	0,00%	8,98%	0,00%
Aminociclitolici	4,03%	0,00%	4,75%	0,31%	14,10%
Penicilline BLR	3,30%	2,91%	4,82%	12,60%	9,46%
Aminoglicosidi	3,18%	19,13%	1,38%	11,93%	0,58%
Amfenicoli	0,00%	0,00%	0,00%	0,00%	1,79%
Tetracicline	0,00%	0,00%	4,81%	19,80%	2,58%
Lincosamidi	4,19%	0,00%	4,75%	0,31%	14,10%

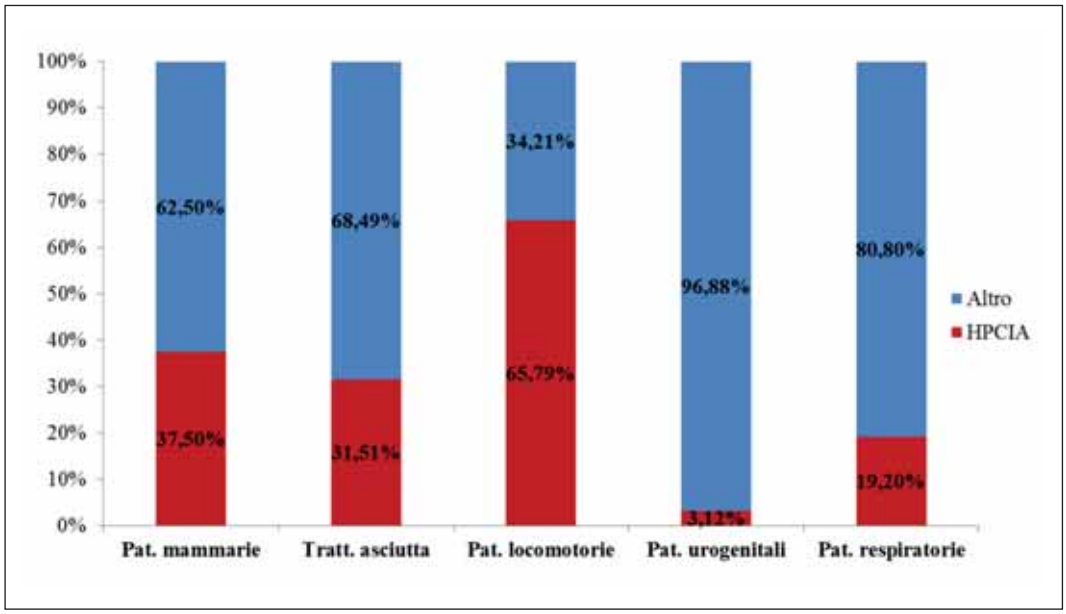

Figura 4
Confronto fra antimicrobici di importanza critica ad alta priorità (*Highest priority Critically Important antimicrobials* - HPCIA) e non-HPCIA per motivo di trattamento, nelle 16 aziende oggetto di studio.

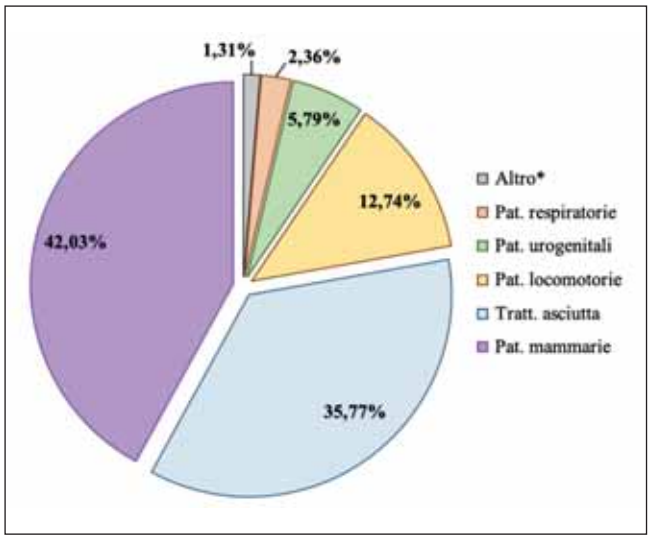

Figura 5 - Cause principali di consumo di antimicrobici, calcolate sulla media ponderata dei giorni/capo per anno (AMU), nelle 16 aziende oggetto di studio.
*Altro: s'intende la % di giorni/capo per anno per la terapia delle patologie enteriche, cutanee, delle setticemie e dei trattamenti antiparassitari.

per le bovine adulte (6/16) e il sovraffollamento delle bovine in lattazione (4/16). Spazi di stabulazione degli animali troppo ridotti, una gestione non adeguata della lettiera o delle cuccette o delle aree di transito degli animali sono state associate ad un aumento delle SCC nel latte di massa, parametro principale per il monitoraggio della sanità della mammella[14]. Infine, per quanto riguarda l'Area C (*animal-based measures*), i punti di maggior carenza sono stati la pulizia degli animali e la presenza di lesioni, infatti, in 10 aziende su 16 più del 20% delle bovine adulte era sporco e in 5 aziende su 16 più del 30% delle bovine adulte presentava lesioni cutanee. L'igiene della bovina è un indicatore importante di benessere, in quanto fornisce informazioni circa la qualità della vita dell'animale e le condizioni igieniche delle strutture d'allevamento, nonché uno dei fattori di rischio più rilevanti per l'instaurarsi di mastiti ambientali[15].

Per quanto riguarda la valutazione della biosicurezza, le principali lacune riscontrate hanno riguardato: la presenza di altre specie animali all'interno del perimetro dell'allevamento (16/16); la mancanza di una distanza adeguata tra gli automezzi in entrata (es. camion che trasportano mangimi o che caricano gli animali vivi) e le aree di stabulazione degli animali (16/16); l'assenza di adeguate procedure di regolamentazione per l'ingresso dei visitatori in azienda, compreso il mancato utilizzo di dispositivi di protezione individuale monouso oppure lavabili e disinfettabili (es. camici, stivali, ecc.) (12/16).
Data la scarsa numerosità del campione non è possibile estendere il dato della biosicurezza all'intero territorio nazionale; tuttavia altri studi suggeriscono, negli allevamenti di bovini da latte, la tendenza ad una conoscenza marginale dei requisiti di biosicurezza e una loro applicazione in maniera saltuaria e discontinua[16]. Altri studi hanno descritto un livello di attuazione delle misure di biosicurezza generalmente basso con ampi margini di miglioramento, nonostante vi sia la consapevolezza negli allevatori dell'importanza di prevenire e controllare le malattie infettive in allevamento. Ad incidere maggiormente è l'assenza di una comprovata efficacia delle pratiche messe in atto e la mancanza di una formazione su questo ambito da parte dei veterinari e di altro personale esperto[17,18].
Per quanto riguarda il consumo di antimicrobici, dalla presente ricerca emerge che oltre il 75% dell'AMU calcolato sia dovuto *in primis* alla terapia delle infezioni mammarie in bovine in lattazione e alla prevenzione di queste nelle bovine in asciutta (Figura 5). Similmente, Kuipers e collaboratori[19] hanno riscontrato un utilizzo dell'antimicrobico per le medesime finalità quasi sovrapponibile (circa il 70% del consumo complessivo). La mastite è considerata la seconda patologia più rilevante in grado di incidere negativamente sul benessere delle bovine da latte, dopo i problemi podali[20], ma è anche quella per cui il ricorso alla terapia antimicrobica risulta eccessivo e inutile. Ad esempio, per quanto concerne la cura in lattazione, è stato evidenziato che circa il 50% dei trattamenti siano inutili[21].
Nella valutazione del consumo di antimicrobici, desta preoccupazione il frequente ricorso agli HPCIA (Figura 3), in particolare a cefalosporine di IV e III generazione. Le cefalospo-

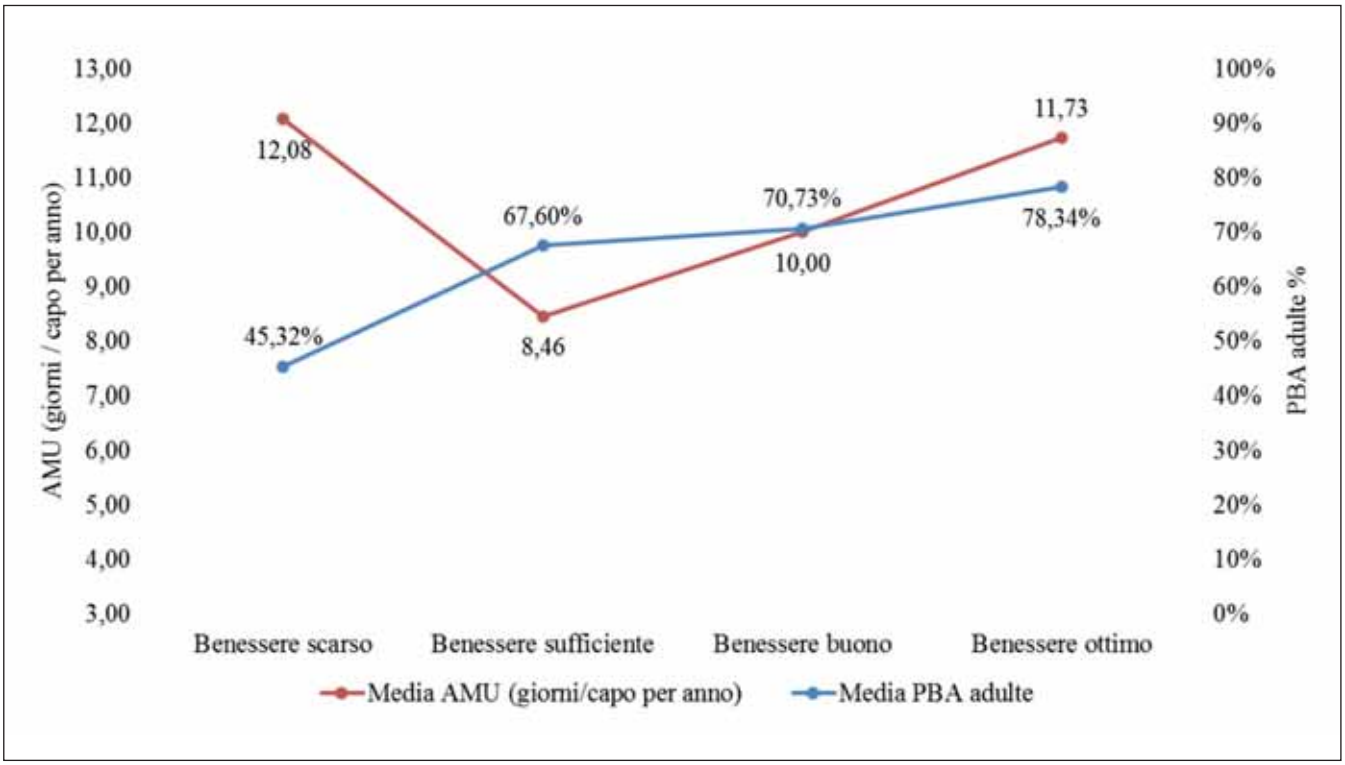

Figura 6 - Confronto tra le medie relative al consumo di antimicrobici [AMU (giorni/capo per anno)] e il Punteggio di Benessere Animale delle bovine adulte (PBA_{adulte}) nelle 16 aziende oggetto di studio, categorizzate in base ai quartili del PBA_{adulte}.

rine di IV generazione sono state impiegate, principalmente, per il trattamento preventivo delle asciutte (29,7%) e per le mastiti nelle bovine in lattazione (13,4%); quelle di III generazione per la cura delle patologie locomotorie (64,5%) e, in misura minore, per le mastiti (5,0%) (Tabella 3). Tuttavia, tali risultati dovrebbero essere interpretati con cautela, tenendo presente la ridotta numerosità campionaria. Il consumo di HPCIA risulta comunque più basso rispetto ad uno studio sul consumo di antimicrobici in 248 stalle da latte austriache, che ha riportato un consumo di HPCIA di circa il 55% per la terapia delle patologie mammarie[22].

Uno studio recente[23] ha, tuttavia, evidenziato la possibilità di ridurre il consumo di antimicrobici contestualmente ad una cessazione completa dell'uso dei HPCIA, mantenendo un adeguato livello di benessere animale, grazie a strategie proattive di pianificazione della sanità della mandria e di gestione dei trattamenti antimicrobici.

Riguardo al considerevole utilizzo di cefalosporine di IV generazione per il trattamento profilattico alla messa in asciutta, osservato nel presente studio, possono essere fatte alcune considerazioni. Sebbene la terapia sistematica riduca l'incidenza di mastiti nella lattazione successiva, questo tipo di gestione è stato fortemente messo in discussione dalla Commissione Europea, che chiede di abbandonarlo a favore di misure alternative[24]. Per rispondere a questa esigenza è stata riconsiderata l'adozione del trattamento selettivo in asciutta: tale pratica, però, se non eseguita in ottimali condizioni di igiene, di management aziendale e con adeguato monitoraggio routinario della sanità della mammella, potrebbe favorire l'instaurarsi di mastiti durante la lattazione successiva[25].

Dall'analisi dei risultati esposti, non emerge una correlazione statisticamente significativa fra l'AMU e il PBA_{adulte}. Ciononostante, l'azienda con PBA_{adulte} peggiore ha un AMU doppio rispetto a quella migliore. Inoltre, le 3 aziende peggiori hanno consumato in media circa il 20% di antimicrobico in più rispetto alle 3 migliori (Tabella 2). Il campione ridotto, l'elevata similitudine fra le aziende delle condizioni relative alla sanità della mammella (nessuna, a parte una, eccede le 300.000 cell/ml di MGBTSCC e nessuna supera le 50.000 ufc/ml di MGCBT) (Tabella 1) congiuntamente ad un motivo di consumo indirizzato principalmente alla terapia dei problemi mammari, rappresentano i motivi per i quali, plausibilmente, non sono state riscontrare correlazioni significative tra il benessere animale e l'AMU. Questo risultato si riflette anche nell'andamento dei due parametri come illustrato in Figura 6.

Per quanto riguarda la biosicurezza, che rappresenta uno strumento importantissimo per ridurre l'ingresso o la circolazione di patogeni in allevamento, non sono emerse correlazioni statisticamente significative con l'AMU. Tuttavia, trattandosi di un numero esiguo di allevamenti, il risultato ottenuto andrebbe approfondito con un'indagine più ampia sul territorio.

CONCLUSIONI

Il sistema ClassyFarm si è dimostrato uno strumento promettente nel fornire importanti informazioni sull'uso degli antimicrobici, sulle condizioni di vita delle bovine da latte e sul livello di biosicurezza delle aziende oggetto di studio. Nell'ottica della riduzione del consumo di antimicrobici negli allevamenti di bovine da latte, l'utilizzo di questo sistema integrato permetterebbe di effettuare analisi precise dei dati, consentendo ad allevatori e veterinari di comprendere al meglio i punti critici su cui agire per razionalizzare l'impiego degli antimicrobici, migliorare il benessere degli animali e la biosicurezza degli allevamenti. La valutazione dell'AMU, attraverso il metodo delle DDDAit (più preciso rispetto agli in-

dicatori basati sulla massa) preparerebbe l'Italia all'adozione dei futuri standard comunitari basati sulle DDDvet, i cui principi sono già stati stabiliti dall'EMA[7].
L'applicazione su larga scala del sistema permetterà di avere dati da un numero elevato di allevamenti italiani in modo da approfondire i risultati ottenuti da questo studio preliminare.

RINGRAZIAMENTI

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Antimicrobial usage, animal welfare and biosecurity in 16 dairy farms in Lombardy

SUMMARY

The present study reports the preliminary results obtained from the on-field application of the new Italian integrated system "ClassyFarm" for the risk-based categorization of dairy farms in relation to animal welfare, biosecurity and antimicrobial usage and it investigates the relationships between these 3 aspects of the farming system. Over the three year-period 2016-2018, sixteen Italian loose housing dairy farms were assessed using this integrated approach.
Animal welfare and biosecurity levels were determined applying the checklist developed by the Italian Reference Centre for Animal Welfare (CReNBA) and included in the ClassyFarm system. The antimicrobial usage (AMU), defined as the number of days in which an animal is potentially exposed to an antimicrobial treatment during a year, was calculated using the Defined Daily Dose Animal for Italy (DDDAit), based on the Italian summaries of product characteristics.
In the sixteen investigated farms, the average animal welfare level was 67.98% (range 45.32%-81.69%), the average biosecurity level was 45.86% (range 21.41% - 71.56%) and the average AMU was 10.57 days/cow per year. Antimicrobials in cows were mostly used for udder problems (42.03%), dry cow therapies (35.77%), locomotion problems (12.74%), urogenital diseases (5.79%) and respiratory diseases (2.36%). Penicillins, third- and fourth-generation cephalosporins were the most used classes of antimicrobials. The most common routes of administration were the injectable route (43.57%) and the intramammary route (34.67% in dry cows and 20.34% in lactating cows, respectively).
No statistically significant correlations (Spearman's rank correlation coefficient) were identified between AMU and the other variables (animal welfare level and biosecurity level), however, further investigations on a larger sample of farms are needed to confirm the obtained results.
The integrated system "ClassyFarm" turned out to be a very promising tool for Italian dairy farms, in order to monitor and enhance animal welfare and biosecurity levels and to support a more rational antimicrobial usage, satisfying National and European demands for the reduction of the antimicrobial usage.

KEY WORDS

Dairy cow welfare; welfare assessment; antimicrobial usage; defined daily dose.

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SOCIETÀ ITALIANA VETERINARI PER ANIMALI DA REDDITO

ASSOCIAZIONE FEDERATA ANMVI

CONVEGNO NAZIONALE

PATOLOGIE METABOLICO-NUTRIZIONALI NELLA BOVINA DA LATTE: SVILUPPI SCIENTIFICI E NUOVI APPROCCI DIAGNOSTICO-TERAPEUTICI

Cremona, 2 Aprile 2020

Palazzo Trecchi - Via Trecchi, 20

OBIETTIVI

Verranno presentante le recenti acquisizioni scientifiche sulla fisiopatologia delle principali patologie metabolico-nutrizionali del periodo di transizione della bovina da latte (acidosi ruminale e chetosi), dando spazio in particolare agli approfondimenti sugli approcci diagnostici e sulla gestione clinica.

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MATTEO GIANESELLA
Ordinario di Clinica Medica Veterinaria, Università degli Studi di Padova

PROGRAMMA SCIENTIFICO

13:45 Saluto di Mario Facchi e inizio lavori

14:00 **Acidosi ruminale nella bovina da latte. Problema vecchio ma con sempre nuove acquisizioni tecnico-scientifiche: facciamo il punto**
Matteo Gianesella

15:45 Discussione

16:00 Pausa caffè

16:15 **Chetosi e Lipidosi nella bovina da latte. Utilizzo di marker biochimici e analisi di imaging ecografico utili ai fini terapeutici**
Enrico Fiore

17:45 Discussione

18:00 Chiusura lavori

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In collaborazione con IZSLER

Energy metabolism indicators and body condition in peripartal period of Alpine goats

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SUMMARY

The investigation was performed on two groups of primiparous and multiparous healthy dewormed Alpine dairy goats (25 each) during peripartal period. Blood samples were collected (jugular venipuncture) 10-15 days before and 10-15 and 30 days after the parturition into BD SST-II Advance (3.5 mL) and BD NaF 3.0 mg Na_2EDTA 6.0 mg (2 mL) vacutainers, cooled and centrifuged (1500 r/min, 15 minutes and ≤1300 r/min, 10 minutes, respectively). Glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) concentrations in blood sera were determined using A15 automatic spectrophotometric analyzer (*Biosystem, Spain*). Simultaneously, body condition scoring (BCS) was performed by Villaquiran et al. (2007) method. The obtained data were analyzed by IBM SPSS statistics 21.

The glucose concentration inclined to increase in both groups. Differences between glucose levels were significant ($P<0.05$) 15 days before and 15 days after, as well as 15 and 30 days after the parturition, and very significant ($P<0.01$) 15 days before and 30 days after the parturition.

The BHBA blood levels significantly differed 15 days before and 30 days after and 15 and 30 days after the parturition ($P<0.05$). BHBA concentration peaked at week 2 postpartum, following the increase of NEFA, providing the substrate for BHBA synthesis. NEFA levels significantly ($P<0.05$) differed 15 days before and 15 days after the parturition. Goats' BCS ranged from 2 to 4 and significantly depended on glucose ($r=0.392$; $P<0.05$) and BHBA ($r=0.317$; $P<0.05$) level 15 days before parturition. BCS 30 days postpartum very significantly depended on the glucose level ($r=0.450$; $P<0.01$), significantly higher than the concentration of BHBA ($r=0.351$; $P<0.05$) and NEFA concentration ($r=-0.304$; $P<0.05$). BCS 15 days before parturition did not depend on the NEFA concentration. Fifteen days after the parturition BCS did not statistically depend on the observed indicators.

Obtained data suggest that knowledge of BCS and energy indicators levels may be very useful in research and practice in order to appreciate energy metabolism of pregnant and lactating dairy ruminants, particularly dairy goats. These data are poorly documented for goats, but they can reveal early pathological metabolic changes in transiting female goat organism, enabling successful prophylactic, as well as, therapeutic intervention.

KEY WORDS

Body condition, energy metabolism, goat, parity, peripartal period.

INTRODUCTION

Pregnancy imposes a substantial cost to the animal, because total requirements for nutrients at the end of pregnancy are about 75% greater than in a no pregnant animal of the same weight[1]. During the transition period (3 weeks before to 3 weeks after parturition) pregnant ruminants must adapt their metabolism to the new and much higher demands for parturition and lactogenesis[1,2,3], and to a different diet in order to meet their new requirements[4], which results in a negative energy balance[5]. It has been demonstrated that nutritional management in the early dry period is important for maintaining the health and productivity of transition cows[2]. After parturition, the demands for glucose, amino acids and fatty acids due to milk production, are 2-5 times higher than pre-partum requirement in ruminants[3,6,7,8]. This is characterized by fat mobilization and elevation of circulating concentrations of non-esterified fatty acids (NEFA)[9], which in most cases is paralleled by an increased production of β-hydroxybutyrate (BHBA) and other ketone bodies[10]. However, when females enter into this period of important metabolic challenges due to imbalance between demands and supply of nutrients without receiving proper care, the possibilities of developing metabolic and/or nutritional disorders become higher, as verified by Souto *et al.*[11].

Glucose is the primary source of energy for the body's cells and the only energy source for the brain and nervous system. A continuous supply must be available and a more or less constant level of glucose must be maintained in the blood. After feeding, the majority of circulating glucose comes from the diet; during fasting, gluconeogenesis and glycogenolysis maintain glucose concentrations. Very small amount of glu-

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cose could be found in the diet as glucose, but as more complex carbohydrates that are broken down to monosaccharides through the digestive process[12].

These metabolic changes and adaptations modify the concentration of blood indicators that are related to the development of the metabolic profile of female. Thus, blood biochemical parameters are the most important indicator used in the determination of the energy, protein, enzymatic, hormonal and mineral profiles, as well as assessing nutritional status, milk production and animal health[13].

Routine scoring of the body condition of dairy animals can help detect potential problems that might cause a decrease in milk production. Body fat reserve in dairy goats bears importance in terms of milk production, fertility, feed consumption and general health of the animal. Sejian *et al.*[14] suggested a positive correlation between BCS at mating and reproductive performance. According to Soares *et al.*[15], higher concentrations of BHBA were observed at the beginning of lactation ($P<0.001$) in relation to parturition and the end of pregnancy. Higher concentrations of BHBA during early lactation were also reported by Sadjadian *et al.*[16] in the healthy dairy goats. Observed high BHBA concentration at parturition with a gradual decrease up to the 8th week of lactation in Alpine goats with different degrees of body condition score related this finding to the use of this metabolite by the mammary gland for the milk fat synthesis. Previous studies reported different BHBA evolution in ewes and dairy cow that showed the highest concentrations before the parturition and *post-partum* respectively[17].

Soares *et al.*[15] found that there was a gradual increase of NEFA concentrations at the end of gestation, reaching a peak at parturition (0.5 ± 0.38 mmol/L; $P<0.001$), and then a subsequently gradual decrease of its concentration during lactation, confirming that the increase of NEFA concentration in *pre-partum* as well as its peak at parturition is due to the high energy demand in the final third of gestation, rapid growth of foetuses and the mammary gland development, confirmed by Barbosa *et al.*[18].

According to Magistrelli and Rosi[19], increase of glycaemia was observed at parturition ($P=0.0079$), during late pregnancy and early lactation in Saanen goats in the peri-partum period. Blood parameters and milk composition alterations are crucial to predict the energy balance status of buffaloes and therefore other ruminants in order to improve their management and feed intake during the transition period[3]. Transition period is an important metabolic challenge to high-yielding dairy cows. Data on these indicators are very useful in order to prevent the outset of nutritional imbalance that typically occurs in high production dairy cows[7]. On the other hand, biochemical attributes during different metabolism statuses generally have not been reporting routinely in goats[13,16]. In light of the presented data, the objective of this study was to evaluate the adaptive changes of the energy biochemical profile of healthy dairy goats of different parities during the peripartal period and its relationship to goats' body condition.

MATERIALS AND METHODS

Investigation took place at a commercial farm in Veliki Gaj (45°17'05"N 21°10'13"E, 80 m a.s.l.) in South Banat District of Serbia. When the study began, there were 115 healthy dairy goats, 54 primiparous goats, 146 kids and 5 bucks on the farm. The study was conducted on 50 clinically healthy, Alpine breed goats, divided in two groups of 25 each, regarding parity (25 primiparous and 25 multiparous) in two pens, in the period from January until March. At kidding, 5 primiparous goats had twins, while other 20 of them gave birth to a single kid. In the group of 25 multiparous goats, one triplet of kids was born, as well as 21 pair of twins and 3 single newborn kids. All procedures with animals were performed according to our institutional guidelines for animal research and principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other (Official Daily N.358/1-358/6, 18, December 1986). According to the health scheme, the goats were dewormed twice a year, based on the results of the parasitological examination of the faeces, 90 days had passed since the last deworming.

The goats were fed two times a day with alfalfa hay *ad libitum* and 1.5 kg of concentrate (16% of crude protein and 1438.42 kcal/kg Dry Matter min.) during milking. Thirty days after kidding, the average daily milk yield in the groups of primiparous and multiparous goats were 2.4 kg and 4.2 kg, respectively.

Blood samples were collected by jugular venipuncture from all observed goats between 9:00 and 11:00 a.m., after morning feeding, 10-15 days before and 10-15 and 30 days after the delivery. Samples were collected into appropriate vacutainers BD SST-II Advance (3.5 mL) and BD NaF 3.0 mg Na_2EDTA 6.0 mg (2 mL) kept in a cool place and then vacutainers were centrifuged at 1500 r/min during 15 minutes and ≤1300 r/min for 10 minutes, respectively, in order to be prepared for further procedures. Analyses of glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) concentrations were performed using A15 automatic analyzer (spectrophotometric - Random Access Analyzer, working set 340 - 900 nm, *Biosystem Spain*). Body condition scoring (BCS) was carried out using method of Villaquiran *et al.*[20], where palpation and observation of three anatomical regions (lumbar region, breastbone and chest) were used. The score was expressed numerically in the scale from 1 to 5. Body condition was evaluated at the same time when blood samples for analysis were taken.

Descriptive statistics, t-test and coefficient of correlation were used for statistical analysis. In addition, statistical significance of differences of all examined parameters were determined by means of the one way ANOVA, followed by the Tukey HSD test. Data were expressed as means ± standard error. Significance level was set at $P \leq 0.05$. Statistical analysis was performed using the SPSS Statistics 21 Software, CA, USA.

RESULTS

The mean values, variability and maximum and minimum values of the concentration of glucose BHBA and NEFA in the blood serum of tested goats and BCS, are shown in Table 1.

Mean values of glucose in the goat's blood of the during the trial ranged from 2.72 mmol/L 15 days before the parturition to 3.61 mmol/L 30 days after the parturition in the primiparous goats, while in the multiparous these values ranged from 3.44 mmol/L 15 days before parturition up to 3.76 mmol/L 30 days after parturition. For primiparous goats, the glucose concentration values ranged from 2 to 5.2 mmol/L,

Table 1 - Concentration of glucose, BHBA and NEFA in goat blood and body condition score.

Parity		$\bar{\chi}$				Sd				Xmax-Xmin				Cv (%)			
		Glucose (mmol/L)	BHBA (mmol/L)	NEFA (mmol/L)	BCS (1-5)	Glucose	BHBA	NEFA	BCS	Glucose (mmol/L)	BHBA (mmol/L)	NEFA (mmol/L)	BCS (1-5)	Glucose	BHBA	NEFA	BCS
PRIMI	- 15 d	2.72	0.28	0.12	3.08	0.28	0.09	0.09	0.37	3.4 - 2.3	0.5 - 0.1	0.3 - 0	4 - 2.5	10.29	33.54	70.92	12.11
	+ 15 d	3.09	0.25	0.27	2.90	0.61	0.12	0.17	0.32	4.3 - 2.0	0.5 - 0.1	0.6 - 0	4 - 2.5	19.71	47.40	63.47	11.13
	+ 30 d	3.61	0.31	0.28	2.84	0.69	0.14	0.26	0.43	5.2 - 2.5	0.7 - 0.1	1.4 - 0	3.5 - 2	19.24	46.79	93.01	15.01
	Σ	3.14	0.28	0.22	2.94	0.66	0.12	0.20	0.38	5.2 - 2.0	0.7 - 0.1	1.4 - 0	4 - 2	16.41	42.58	75.80	12.75
MULTI	- 15 d	3.44	0.31	0.17	3.50	0.60	0.06	0.22	0.43	5.1 - 2.1	0.4 - 0.2	0.49 - 0	4 - 2.5	17.57	20.79	129.43	12.37
	+ 15 d	3.61	0.47	0.23	2.92	0.44	0.17	0.25	0.40	4.8 - 3.2	1.0 - 0.2	0.9 - 0	3.5 - 2	11.88	36.96	110.75	13.70
	+ 30 d	3.76	0.58	0.08	3.12	0.46	0.26	0.10	0.36	5.44 - 3.2	1.4 - 0.3	0.46 - 0	3.5 - 2.5	12.16	46.01	126.63	11.59
	Σ	3.63	0.45	0.16	3.18	0.52	0.21	0.21	0.46	5.44 - 2.1	1.4 - 0.2	0.9 - 0	4 - 2	13.87	34.59	122.27	12.55
Total	- 15 d	3.08	0.29	0.15	3.29	0.59	0.08	0.17	0.45	5.1 - 2.1	0.5 - 0.1	0.49 - 0	4 - 2.5	19.12	27.54	114.09	13.76
	+ 15 d	3.39	0.36	0.25	2.91	0.61	0.18	0.21	0.36	4.8 - 2.0	1.0 - 0.1	0.9 - 0	4 - 2	17.94	51.12	86.31	12.37
	+ 30 d	3.69	0.44	0.18	2.98	0.59	0.25	0.22	0.42	5.44 - 2.5	1.4 - 0.1	1.4 - 0	3.5 - 2	15.93	56.74	121.50	13.96
	Σ	3.39	0.36	0.19	3.06	0.64	0.19	0.20	0.44	5.44 - 2.0	1.4 - 0 1	1.4 - 0	4 - 2	17.66	45.13	107.30	13.36

PRIMI - primiparous goats, **MULTI** - multiparous goats, Σ - total, **- 15 d** - 15 days before parturition, **+ 15 d** - 15 days after parturition, **+ 30 d** - 30 days after parturition.

and in multiparuos, 2.1 to 5.44 mmol/L. The variability of the glucose concentration was not high and ranged from 10.29% 15 days before to 19.71% 15 days after the parturition in primiparous, while in the multiparous it ranged from 11.88% 15 days to 17.57% 15 days before the parturition.

Mean BHBA concentration in goat's blood during the trial ranged from 0.25 mmol/L 15 days after parturition to 0.31 mmol/L 30 days after parturition in primiparous goats, while in multiparous these values ranged from 0.31 mmol/L 15 days before parturition up to 0.58 mmol/L 30 days after the parturition. The BHBA concentrations in the primiparous goats ranged from 0.1-0.7 mmol/L, and in the multiparous 0.2-1.4 mmol/L. The variability of the BHBA concentration ranged from 33.54% 15 days before to 47.40% 15 days after the parturition in the primiparous, while in multiparous ranged from 20.79% 15 days before to 46.01% 30 days after the parturition.

Mean NEFA levels in goat's blood during the trial ranged from 0.12 mmol/L 15 days before the parturition to 0.28 mmol/L 30 days after the parturition in the primiparous goats, while in multiparous these values ranged from 0.08 mmol/L 30 days after parturition up to 0.23 mmol/L 15 days after parturition. For primiparous goats, NEFA concentration values ranged from 0 to 1.4 mmol/L, and in multiparous 0-0.9 mmol/L. The variability of the NEFA concentration was high and ranged from 63.47% 15 days before to 93.01% 30 days after the parturition in the primiparous, while the multiparous ranged from 110.75% 15 days after to 129.43% 15 days before the parturition.

The mean values of BCS in the examined periods ranged from 2.84 30 days after parturition to 3.08 15 days before parturition in primiparous goats, while in multiparous these values ranged from 2.92 15 days after parturition to 3.50 15 days before parturition. In primiparous goats, BCS ranged from 2 to 4, which was the case with multiparous, too. The variability was lowest in primiparous 15 days after the parturition (11.13%) and the highest 30 days after the parturition (15.01%), also in primiparous goats.

Between two groups of goats (primiparous and multiparous), very significant differences in glucose levels were found 15 days before and 15 days after parturition, in the level of BHBA 15 and 30 days after the parturition, as well as in the NEFA level 30 days after parturition (Table 2).

In other cases, the differences were not significant. In addition, a very significant difference in the BCS 15 days before the parturition and a significant difference 30 days after the parturition were found. No significant differences were found 15 days after the parturition.

Factors parity and the time of sampling have very significantly influenced the glucose and BHBA levels and BCS, and significantly on NEFA level. The influence of the interaction of these two factors (parity x time of sampling) on glucose level and BCS was significant, while on BHBA and NEFA levels was very significant (Table 3).

According Table 4, the results of the Tukey HSD test indicated that the glucose level established 15 days before the parturition significantly differed from the level established 15 days after the parturition and very significantly from the level of 30 days after the parturition. The level of glucose 15 days after the parturition significantly differed from the level established thirty days after the parturition. The levels of BHBA in the blood significantly differed 15

Table 2 - Primiparous and multiparous goats glucose, BHBA, NEFA and BCS levels differences.

Term of measurement	Glucose		BHBA		NEFA		BCS	
	t-test	p	t-test	p	t-test	p	t-test	p
15 days before	-5.357**	0.000	-1.422^{ns}	0.162	-0.984^{ns}	0.333	-3.674**	0.001
15 days after	-4.051**	0.000	-5.203**	0.000	0.657^{ns}	0.514	-0.195^{ns}	0.847
30 days after	-0.933^{ns}	0.356	-4.442**	0.000	3.517**	0.001	-2.504*	0.016

^{ns} - p>0.05, * - p<0.05, ** - p<0.01

Table 3 - Parity and time of sampling significance regarding glucose, BHBA, NEFA and BCS.

Indicator	Glucose		BHBA		NEFA		BCS	
Values	F	p	F	p	F	p	F	p
Parity	32.105**	0.000	45.563**	0.000	3.943*	0.049	14.347**	0.000
Time of sampling	16.243**	0.000	11.391**	0.000	3.483*	0.033	13.583**	0.000
Interaction	3.886*	0.023	7.864**	0.001	5.003**	0.008	3.421*	0.035

* - p<0.05, ** - p<0.01

Table 4 - Tukey HSD test results for glucose, BHBA and NEFA levels and BCS.

Indicator	Glucose	BHBA	NEFA	BCS
Term of sampling	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$
15 days before	3.08 ± 0.08^{c}	0.29 ± 0.01^{b}	0.15 ± 0.02^{b}	3.29 ± 0.06^{a}
15 days after	3.39 ± 0.09^{b}	0.36 ± 0.02^{b}	0.25 ± 0.03^{a}	2.91 ± 0.05^{b}
30 days after	3.69 ± 0.08^{a}	0.44 ± 0.03^{a}	0.18 ± 0.03^{ab}	2.98 ± 0.06^{b}

Means followed by different letters differ significantly at P<0.05.

days before and 30 days after the parturition, and a significant difference was found between the levels 15 and 30 days after the parturition. Fifteen days before and 15 days after the parturition the differences were not significant. The levels of NEFA in the blood of examined goats significantly differed 15 days before and 15 days after the parturition. In other cases, the differences were not significant.

BCS significantly differed 15 days before the parturition compared to 15 days after and 30 days after the parturition. The differences were not significant 15 days after and 30 days after the parturition.

The correlation between concentrations of glucose, BHBA, NEFA and BCS is also calculated. BCS 15 days before parturition statistically very significantly depended on the glucose concentration (r = 0.392; P<0.01), and statistically significant depended on the BHBA concentration (r = 0.317; P<0.05) and did not depend on the NEFA concentration. Fifteen days after the parturition BCS did not statistically depend on the observed indicators. Thirty days after the parturition BCS statistically very significantly depended on the concentration of glucose (r = 0.450; P<0.01), and statistically significant depended on the concentration of BHBA (r = 0.351; P<0.05) and on the NEFA concentration (r = -0.304; P<0.05). Fifteen days after parturition BHBA and NEFA concentrations very significantly depended on the glucose concentration (r = 0.485 and r = -0.387; P<0.01, respectively). Thirty days after parturition BHBA and NEFA concentrations very significantly depended on the glucose concentration (r = 0.369 and r = -0.383; P<0.01, respectively) and NEFA concentration very significantly depended on the BHBA concentration (r = -0.434; P<0.01).

DISCUSSION

An increase of glucose concentration at parturition is due to the high concentration of glucocorticoid hormones such as cortisol, which promotes an increase in hepatic glycogenolysis and gluconeogenesis from glucose precursors[16,19]. The result obtained is consistent with the results of Radin *et al.*[21], who found significantly higher glucose concentrations in foetuses and lower parities compared to older animals two and four weeks after parturition (P<0.01). The same authors report lowering blood glucose levels in older animals, unlike primiparous goats. Evolution of glucose concentration during late pregnancy and early lactation was similar observed by other studies in Saanen goats in the peri-partum period[19]. Certain studies have reported a decrease in glycaemia in the first weeks of lactation, especially in high producing dairy goats, related to high demand for milk lactose synthesis[16,19]. Other studies also reported similar glycemia in sheep[22] and in dairy cows[17]. Moreover, a recent study has reported that the regulation of glucose homeostasis changes at different physiological stages. Also, an additional elevation of BHBA beyond the metabolic adaptation after parturition might change glucose concentration in early-lactation dairy cows[17]. Slightly different results were obtained by Soares *et al.*[15], who found that the glucose level was the highest during the kidding and then decreased (P<0.05). Antunović *et al.*[13] recorded a significant drop in the level of glucose in the observed period, and later as lactation progresses.

The BHBA concentration tended to increase in our study. Synthesized from fatty acids during energy deficiency, BHBA composes the main part of the ketone bodies[23]. If the concentrations of the ketone bodies in body fluids exceed a certain level, the adaptability of metabolism is exceeded and whole-body homeostasis cannot be maintained[24]. The low level of serum BHBA recorded at all stages of lactating cows suggested that they have adapted for the state of negative energy balance[25].

BHBA concentration increased after parturition and peaked at week 2 postpartum, following the increase of NEFA; this suggests that NEFA provides the substrate for BHBA synthesis. This increase in BHBA concentration reveals incomplete oxidation of NEFA in the tricarboxylic acid cycle during negative energy balance[26]. Obtained results were in accordance with the results of Sadjadian *et al.*[16] indicated that the changes in BHBA concentrations were between 134 and 375 μmol/L, with a low number of does with BHBA concentrations above 1,000 μmol/L. This increase of the BHBA concentration in early lactation is due to the high energy requirement in organisms such as cattle with a high milk yield[27]. However, despite this increased concentration of BHBA observed in this study, mean values of this variable were within the reference interval for the species (≤0.8 mmol/L, according Rook[28]).

NEFA concentrations grew in primiparous goats as in multiparous decreased. For primiparous goats, NEFA concentration values ranged from 0 to 1.4 mmol/L, and in multiparous 0-0.9 mmol/L. The variability of the NEFA concentration was high, especially in the case of multiparous goats. Similarly, Soares *et al.*[15] found that the NEFA level was the highest during kidding and subsequently declined ($P<0.05$).

During early lactation ruminants can mobilize considerable amounts of body fat to maintain milk production[29,30]. Authors proved the link between NEFA values and the energy balance in goats. This study predicts a value of 217 μmol/L of NEFA at zero Energy Balance.

Similar to obtained results in this study, NEB (Negative Energy Balance) occurs in dairy Saanen goats during the periparturition period. NEFA concentration reflected a NEB better than BHBA in dairy Saanen goats. In addition, Sadjadian *et al.*[16] found out that the number of does with abnormal NEFA concentrations (≥0.6 mmol/L) was high.

The magnitude of the metabolic challenge during the peripartum period due to the higher energetic demand causes a greater release of NEFA into the bloodstream due to the lipolysis rate that overlaps with the lipogenesis. Part of this metabolite is used as a source of energy by peripheral tissues and another part is metabolized in the liver, being completely oxidized for energy production or partially oxidized to produce ketone bodies or esterified and stored as triglycerides[18]. The NEFA concentrations obtained during this study have not exceeded values considered normal for the species, being to those reported by other authors in clinically healthy goats[31,32]. These results have demonstrated the ability of adaptive mechanisms in order to adjust to the demand situation without developing metabolic disorders in different species of ruminants[16,18,19,22]. According to Eşki *et al.*[26], during the postpartum period, blood NEFA concentration reflects the rate of lipolysis or lipomobilization; that is, NEFA levels model the balance between lipolysis and the reesterification of the fatty acids[23]. Hence, evaluation of plasma NEFA concentrations during the periparturient period should provide insight into the time course of fatty liver development[32]. Blood NEFA concentrations consistently increased 2 weeks prepartum until 2 weeks postpartum and reached a peak at 2 weeks postpartum and then steadily decreased, reflecting the mobilization of body fat[26]. Pirmohammadi *et al.*[33] reported that the plasma NEFA concentrations are useful indicators to monitor the energy status of goats in the last month of gestation.

Animal body condition is considered to be an indicator of body fat reserves, which reflect the production performance of the herd[34]. Under farm conditions BCS is an important tool to assess the adequacy of feeding programmes[35]. When overall body condition starts to decrease in the goats, it is a sign that managerial intervention such as supplemental feeding, deworming or pasture rotation is needed. Conversely, when overall body condition starts to increase in the herd, it is a sign that the producer should reduce supplemental feeding[36]. Goats need to be maintained at a moderate amount of body condition. Therefore, BCS is a useful tool to manage feeding of the herd[37]. The study of Cavestany *et al.*[38] describes this effect of parity (multiparous versus primiparous) and body condition score (BCS) at calving (<3 or 3 or more on scale 1-5), body weight (BW) and metabolic profiles in Holstein cows grazing on improved pastures, confirming that primiparous cows had lower BCS during the early postpartum (PP) period and produced less milk than multiparous. In primiparous cows NEFA concentrations were higher during the early postpartum period; BHBA levels were similar in both categories during this period. Primiparous cows showed a more unbalanced metabolic profile than multiparous cows, reflecting that they are recovering from the loss of BCS after calving with less success. This is supported by research of Pambu[36], who found out that BCS has an effect on blood glucose. Fifteen days after parturition BHBA and NEFA concentrations very significantly depended on the glucose concentration ($r = 0.485$ and $r = -0.387$; $P<0.01$, respectively). According to Sadjadian et al.[16], it appears to be related to high energy demands for lactation, especially in high milk-producing breeds of goats. The mobilization of body reserves entailed a gluconeogenesis mechanism which boosted blood glucose concentrations. According this author, does with BCS 3 were significantly different in blood glucose concentrations from does with BCS 2.

CONCLUSION

According presented and analyzed data, the concentration of glucose had a tendency to increase in both groups of goats. The glucose level established 15 days before the parturition significantly differed from the level established 15 days after the parturition and very significantly from the level of 30 days after the parturition. The level of glucose 15 days after the parturition significantly differed from the level established thirty days after the parturition.

The BHBA concentration tended to increase and the blood levels significantly differed 15 days before and 30 days after the parturition, and a significant difference was found between the levels 15 and 30 days after the parturition. Fifteen days before and 15 days after the parturition, the differences were not significant.

BHBA concentration increased after parturition and peaked at week 2 postpartum, following the increase of NEFA, which suggests that NEFA provides the substrate for BHBA synthesis. The variability of the NEFA concentration was high, especially in the case of multiparous goats.

For primiparous goats, physical fitness estimates ranged from 2 to 4, which was the case with multiparous goats. The variability was lowest in primiparous 15 days after the parturition (11.13%) and the highest 30 days after the parturition (15.01%), also in primiparous goats. In most cases, the BCS has decreased, which is probably a consequence of using body reserves for lactation needs.

Fifteen days prior to parturition BCS was significantly dependent on the glucose concentration ($r = 0.392$; $P<0.05$), as well as on the concentration of BHBA ($r = 0.317$; $P<0.05$) and did not depend on the NEFA concentration. BCS 15 days after the parturition did not statistically depend on the observed indicators. BCS was statistically very significantly dependent on the concentration of glucose ($r = 0.450$; $P<0.01$), significantly higher than the concentration of BHBA ($r = 0.351$; $P<0.05$) and NEFA concentration ($r = -0.304$; $P<0.05$). The mobilization of body reserves entailed a gluconeogenesis mechanism which boosted blood glucose concentrations.
Obtained data suggest that knowledge of BCS and energy indicators levels may be very useful in research and practice in order to appreciate energy metabolism of pregnant and lactating dairy ruminants, particularly dairy goats. These data are poorly documented for goats, but they can reveal early pathological metabolic changes in transiting female goat organism, enabling successful therapeutic intervention.

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The effect of breed on instrumental meat quality traits of weaning kids from Turkish indigenous goat breeds

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SUMMARY

Introduction - Meat quality has always been very important to the consumer due to its can affect consumer preferences. The indigenous goat breeds is an important source of meat production source for Turkey. Nevertheless, meat quality characteristics of Turkish native sheep breeds are virtually unknown for native goat breeds, especially among those that are reared for their meat.
Aim - The aim of the study was to determine the effect of breed on instrumental meat quality characteristics in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles from weaning male kids born to Angora, Hair, Honamli and Kilis Turkish indigenous pure goat breeds.
Materials and methods - Angora (n=6), Hair (n=6), Honamli (n=6) and Kilis (n=6) kids were slaughtered at 3 months of weaning age and Longissimus-dorsi (LD) and Semitendinosus (ST) muscles samples were collected to determine meat quality characteristics. Meat quality characteristics was assessed by instrumental analysis.
Results - A significant breed effect was observed for some instrumental measurements of meat quality (pH, drip loss, cooking loss, frozen-thawing loss, water holding capacity, shear force and color characteristics) in LD and ST muscles ($p<0.05$). Total protein content in LD and ST muscles were similar between kids, but effect of breed on dry matter, ash and intra-muscular fat was significant in LD and ST muscles ($p<0.05$).
Discussion - Meat quality traits of kids born to Turkish native goat breeds differ and this result may be sourced due to variety in growth or development and distribution of fat deposits among breeds studied.
Conclusions - It was concluded that there is a measurable effect of breed on meat quality characteristics and chemical composition in weaning male kids born to Turkish indigenous goat breeds. Differences in meat quality characteristics among breeds may be help production of alternative kid meat or might be used to offer kid meat for consumers with different demand.

KEY WORDS

Goat, native breeds, kid, meat quality, longissimus-dorsi, semitendinosus.

INTRODUCTION

Meat has always been very important food to the consumer due to its essential nutrients[1]. Moreover, quality characteristics of meat from meat-producing animals can affect consumer preferences[2]. With rising income levels in developing countries, the consumption of animal origin protein, especially red meat consumption is increasing day-by-day[3]. Additionally, nowadays consumers prefer meat with better quality such as lean, easy cooked (less tender) and more delicious[4]. Red meat demand has been increased with economic developments in Turkey, but the consumption of goat meat has continuously decreased probably due to low organoleptic quality, low supply and unattainable price in especially kids meat.

Turkey has more than 10 million goats and its known 17 different breeds and types, which well suited to the harsh climatic conditions, poor pasture that are the characteristics of rocky and rugged the hills and uplands areas and resistant to most local diseases[5,6]. Therefore, the native goat breeds have significant potential as an important red meat production source for Turkey. The most commonly raised native goat breeds in Turkey are Angora, Hair, Honamli and Kilis. Therefore, these breeds constitutes nearly 92% of the goat population in the Turkey[5,6]. Angora goat breed is reared for fibre production and the only true producer of mohair in the world, additionally this breed use for milk and meat production[5]. Hair goats are remarkable differences in their body sizes and they are bred mainly for meat and milk production[5]. Honamli and Kilis goats breeds are reared for their meat, milk, and wool[5,6].

Dhanda et al.[7] and Santos et al.[8] reported that breed or genotype is one of the most important factors affecting kids meat quality. Moreover, indigenous breeds constitutes a large part of goatherds for meat production in many Mediterranean countries. Additionally, the breeding of goat is carried out under extensive conditions, in which there is no additional or supplementary feeding practices[5]. Therefore, it is possible to offer meat with different properties such as less tender, lean or not by the determination of meat quality traits obtained from native goat breeds. Knowledge related to carcass

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characteristics and meat quality in low weight weaning kids from Turkish native goat breeds is limited. To take advantage of the different schemes of breed utilization, the carcass and meat quality characteristics of the native breeds should be known. Numerous studies have examined meat quality characteristics of Turkish native sheep breeds[2,9], but there is little data from comparative studies for meat quality of kids from Turkish native goat breed.

The present study was, therefore, conducted to comparatively determine meat quality characteristics in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles from weaning male kids born to Angora, Hair, Honamli and Kilis Turkish indigenous goat breed.

MATERIAL AND METHODS

The experimental procedures were approved by the Local Animal Care and Ethics Committee of Kır ehir Ahi Evran University, Kırşehir, Turkey, ensuring compliance with EC Directive 86/609/EEC for animal experiments. A total of 24 male kids of Angora (n=6), Hair (n=6), Honamli (n=6) and Kilis (n=6) breeds were used as experimental animals. Kids were obtained from the national sheep and goat-breeding project in Ankara (Angora), Tokat (Hair), Antalya (Homanlı) and Kilis (Kilis) provinces of Turkey. Each breed were raised in different locations and all kids were born in same breeding season (between May-August, 2016). Management and feeding procedures for all kids were similar until weaning. Following the kidding period, all kids were kept with their dams indoors in multiple boxes until 90 days of weaning age. Kids were fed with does milk yet we were not able to measure the amount of milk consumed by the kids. Also, some good quality alfa alfa hay were present free by the 3rd week after birth in the barn for the kids. Two weeks onwards kidding, does grazed during daytime and met with the kids at night in the barn in order to allow them to suckle until weaning. Kids were slaughtered in different abattoirs with similar conditions, according to the standard commercial slaughtering procedures, at 90 days of weaning age, which is the usual end-user preference about the slaughter age in the region. None of the kids were fed overnight (approximately 16 h) before the slaughter process. Then, all kids were transported to an abattoir in their location.

Following slaughter, the carcasses of all kids were chilled for 24 h at 4°C. After chilling, approximately 150-200 g muscle samples were collected from the central parts of the mid-section of the whole LD and ST muscles, which were taken from the left side of the carcasses, to determine the meat quality traits. These samples were trimmed of subcutaneous fat and fascia. After homogenizing of muscle samples, dry matter, total protein (N × 6.25), intramuscular fat and ash contents were analyzed according to AOAC[10] (1990) procedures. The water holding capacity and frozen-thawing loss of meat samples were determined as described by Aksoy et al.[1]. Water holding capacity (approximately 25 g meat samples) were determined by the filter-paper press method. Approximately 50 g meat samples from both muscles were vacuum packed and stored -20°C for one week to evaluate thawing loss values. The meat sample packages were thawed under tap water, and then the thawing loss values were expressed as a percentage of initial weight prior to freezing. To determine the drip loss percentage of the meat samples, approximately 50 g of each muscle were vacuum-packaged and stored at 4°C. The drip loss values were measured on the 3rd and 7th days of storage. The muscle samples were put in plastic bags and cooked for 40 min in a water bath with at 70°C constant temperature. Following the cooking step, the samples were cooled under tap water. The cooking loss values were calculated as % of weight loss. Shear force values of cooked samples (cut parallel to the muscle fibres with a cross section of 2 × 2 cm) were determined using a Texture Analyzer, (CT3, Brookfield Co., USA).

The pH value was determined using the meat pH meter with a puncture electrode (Testo 205, Lenzkirch, Germany) at 1 h and 24 h postmortem. Lightness (L*), redness (a*) and yellowness (b*) value of the meat samples were measurement of color by using a Chroma Meter (Konica Minolta CR-410, Minolta Co., Ltd., Osaka, Japan) at 1 h and 24 h postmortem. Chroma [C*, the square root of $(a^{*2} + b^{*2})$] and hue angle [H°, $\tan^{-1}(b^*/a^*)$] were also calculated according to Sen et al.[2] at 24 h post-mortem. Color difference (ΔD) was calculated using the following formula: $\Delta D = [(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2]^{1/2}$, where L_1^*, a_1^* and b_1^* represent color parameters measured at 1 h, and L_2^*, a_2^* and b_1^* represent color parameters measured at 24 h post-mortem[2].

The total protein, ash and intra-muscular fat content was determined as a percentage of dry (samples were retained 12 h at 105°C) meat samples weight. Water holding capacity, drip loss, cooking loss and frozen-thawing loss was determined as a percentage of fresh meat samples weight. Mean pH, color characteristics and shear force data from six measurements of each sample were used in the data analysis.

The statistical analysis was conducted on completely randomized design for traits. The statistical analyses were performed using SPSS 17.0 package program (SPSS, Chicago, IL, USA). Significant differences between means were tested by Duncan's multiple comparison tests. Results were computed as mean ± SE and statistical significance was determined at the level of $p<0.05$.

RESULTS

The pH values, drip loss, cooking loss, frozen-thawing loss, water holding capacity and shear force of LD and ST muscles from male kids born to Turkish indigenous goat breeds are given in Table 1.

The pH values of LD and ST muscles in Hair (except for LD muscle at 24 h postmortem) and Angora kids was higher ($p<0.05$) than Kilis and Honamli at 1 h and 24 h postmortem. Additionally, pH drop from 1 h to 24 h postmortem in LD and ST muscles were relatively higher in Kilis kids compared to other breeds ($p<0.05$). In the present study, there were significant differences among kids born to Angora, Hair, Honamli and Kilis breeds in terms of drip loss, cooking loos, frozen-thawing loss and water holding capacity ($p<0.05$). Angora kids had lower percentage of drip loss in LD muscle, but Kilis kids had higher percentage of drip loss in ST muscle on day 3 compared to other breeds ($p<0.05$). Kilis and Honamli kids had higher percentage of drip loss in LD muscle and also Kilis kids had higher percentage of drip loss in ST muscle on day 7 compared to other breeds ($p<0.05$). Percentage of cooking loss in Hair kids was higher

Table 1 - The pH, drip loss, cooking loss, frozen-thawing loss, water holding capacity and shear force values of Longissimus-dorsi (LD) and Semitendinosus (ST) muscles from male kids born to Turkish indigenous goat breeds.

Traits	Muscles	Kilis	Honamli	Hair	Angora
pH					
1 h	LD	6.10 ± 0.11^{b}	6.14 ± 0.12^{b}	6.57 ± 0.13^{a}	6.99 ± 0.07^{a}
	ST	6.18 ± 0.06^{b}	6.16 ± 0.13^{b}	6.51 ± 0.14^{a}	6.56 ± 0.04^{a}
24 h	LD	5.46 ± 0.01^{b}	5.68 ± 0.14^{b}	6.15 ± 0.07^{ab}	6.64 ± 0.05^{a}
	ST	5.46 ± 0.01^{b}	5.62 ± 0.12^{b}	6.21 ± 0.07^{a}	6.23 ± 0.05^{a}
Drop	LD	0.64 ± 0.11^{a}	0.47 ± 0.04^{b}	0.42 ± 0.02^{b}	0.35 ± 0.07^{b}
	ST	0.72 ± 0.07^{a}	0.54 ± 0.07^{ab}	0.30 ± 0.20^{b}	0.33 ± 0.06^{b}
Drip loss (%)					
3 days	LD	11.17 ± 1.94^{a}	10.81 ± 2.40^{a}	10.91 ± 0.26^{a}	6.07 ± 0.41^{b}
	ST	20.16 ± 0.73^{a}	11.97 ± 0.82^{b}	10.07 ± 1.07^{bc}	7.77 ± 0.89^{c}
7 days	LD	19.82 ± 2.75^{a}	18.21 ± 1.34^{a}	13.71 ± 0.13^{b}	13.26 ± 0.86^{b}
	ST	38.90 ± 11.2^{a}	21.23 ± 1.83^{b}	17.89 ± 1.39^{bc}	16.17 ± 1.11^{bc}
Cooking loss (%)	LD	23.13 ± 1.40^{b}	29.29 ± 3.16^{ab}	34.61 ± 1.44^{a}	22.86 ± 1.49^{b}
	ST	30.44 ± 2.82^{b}	28.49 ± 4.02^{b}	36.84 ± 2.69^{a}	28.95 ± 2.36^{b}
Frozen-thawing loss (%)	LD	6.21 ± 0.74^{a}	5.23 ± 0.52^{a}	6.72 ± 0.39^{a}	3.77 ± 0.53^{b}
	ST	8.67 ± 0.34^{a}	6.34 ± 1.75^{ab}	4.89 ± 0.26^{b}	4.71 ± 0.31^{b}
Water holding capacity	LD	28.18 ± 0.31^{a}	29.20 ± 1.73^{a}	25.99 ± 0.97^{a}	13.38 ± 1.06^{b}
	ST	27.94 ± 0.73^{a}	30.12 ± 0.60^{a}	28.16 ± 1.14^{a}	18.23 ± 0.69^{b}
Shear force (kg/cm^{2})	LD	9.92 ± 0.58^{a}	9.23 ± 0.95^{a}	9.62 ± 0.51^{a}	4.25 ± 0.21^{b}
	ST	15.02 ± 1.35^{a}	13.48 ± 2.57^{a}	12.12 ± 1.85^{a}	7.11 ± 0.89^{b}

$^{a, b, c}$ = The differences indicated by different letters on the same line are significant.

than those of other breeds (except for Honamli kids in LD muscle) in both muscles ($p<0.05$). Angora kids had lower percentage of frozen-thawing loss than those of other breeds in LD muscle ($p<0.05$). Frozen-thawing percentage of Angora kids in ST muscle were similar with Hair and Honamlı kids, but they had lower percentage of frozen-thawing loss than those of Kilis kids ($p<0.05$). Similarly, shear force and water holding capacity values of Angora kids was lower compared to other breeds in both muscles ($p<0.05$).

The color characteristics of LD and ST muscles from male kids born to Turkish indigenous goat breeds are given in Table 2.

In the present study, Angora kids had lower ($p<0.05$) lightness (L*) value than those of other breeds (except for Hair in LD muscle), but Kilis kids had higher ($p<0.05$) L* value than those of other breeds (except for Hair in LD muscle) in LD and ST muscles at 1 h and 24 h postmortem. Hair kids had higher ($p<0.05$) redness (a*) value than those of Kilis in both muscles at 1 h and 24 h postmortem. The yellowness (b*) value of Angora kids were lower ($p<0.05$) than those of other breeds (except for Hair) in LD muscle, but Hair kids had higher ($p<0.05$) b* value than those of other breeds (except for Honamli) in ST muscle at 1 h postmortem. Angora kids had lower ($p<0.05$) b* value than those of other breeds in both muscles at 24 h postmortem. Chroma (C*) value of Hair kids were higher ($p<0.05$) than those of other breeds (except for Honamli) in LD muscle at 24 h postmortem. Similarly, Hair and Honomli kids had higher ($p<0.05$) C* value than those of Kilis and Angora in ST muscle at 24 h postmortem. Angora kids had lower hue angle (H°) value than kids born to other breeds (except for Hair in ST muscle) in both muscles at 24 h postmortem. Meat color difference (ΔD) of Angora kid were lower ($p<0.05$) in LD muscle compared to other breeds (except for Honamli), but Kilis kids had higher ($p<0.05$) ΔD value in ST muscle compared to other breeds at 24 h postmortem.

The chemical composition of LD and ST muscles from male kids born to Turkish indigenous goat breeds are given in Table 3. There were significant differences among kids born to Turkish pure goat breeds in terms of chemical composition in LD and ST muscles. Hair and Angora kids had higher ($p<0.05$) percentage of dry matter than those of Kilis and Honamli in LD muscle at 24 h postmortem. Similarly, Hair kids had higher ($p<0.05$) percentage of dry matter than those of Kilis in ST muscle at 24 h postmortem. Honamli kids had higher ($p<0.05$) percentage of ash compared to kids born to other breeds in both muscles at 24 h postmortem. There were no significant differences among kids in terms of percentage of total protein in LD and ST muscles at 24 h postmortem. Angora kids had higher ($p<0.05$) intra-muscular fat content in both muscles compare to kids born to other breeds (except for Kilis in ST muscle) at 24 h postmortem.

DISCUSSION

Breed effects on meat quality traits defined in the present study may be due to differences in breed specific differences in growth or development and distribution of fat deposits among breeds studied.

Table 2 - Color characteristics of Longissimus-dorsi (LD) and Semitendinosus (ST) muscles from male kids born to Turkish indigenous goat breeds.

Traits	Muscles	Kilis	Honamli	Hair	Angora
Lightness (L*)					
1 h	LD	45.60 ± 0.59^{a}	44.69 ± 0.73^{a}	43.22 ± 0.76^{ab}	41.53 ± 0.71^{b}
	ST	47.13 ± 0.49^{a}	46.26 ± 0.97^{ab}	44.70 ± 0.29^{b}	44.65 ± 0.34^{b}
24 h	LD	49.49 ± .98^{a}	46.85 ± 0.50^{b}	47.05 ± 0.71^{ab}	42.24 ± 0.54^{c}
	ST	53.10 ± 0.56^{a}	48.86 ± 0.76^{b}	48.31 ± 0.52^{b}	46.56 ± 0.53^{b}
Redness (a*)					
1 h	LD	16.78 ± 0.37^{b}	17.89 ± 0.26^{ab}	18.34 ± 0.30^{a}	17.93 ± 0.57^{ab}
	ST	16.75 ± 0.26^{b}	18.05 ± 0.34^{ab}	19.08 ± 0.47^{a}	18.39 ± 0.89^{ab}
24 h	LD	18.73 ± 0.44^{b}	19.36 ± 0.28^{ab}	20.35 ± 0.35^{a}	19.48 ± 0.10^{ab}
	ST	17.54 ± 0.37^{b}	19.67 ± 0.34^{a}	20.00 ± 0.33^{a}	18.44 ± 0.2^{ab}
Yellowness (b*)					
1 h	LD	6.14 ± 0.14^{a}	6.03 ± 0.34^{a}	5.51 ± 0.18^{ab}	5.00 ± 0.17^{b}
	ST	5.25 ± 0.14^{b}	5.85 ± 0.17^{ab}	6.26 ± 0.45^{a}	5.19 ± 0.18^{b}
24 h	LD	10.46 ± 0.34^{a}	9.57 ± 0.30^{a}	10.07 ± 0.31^{a}	8.42 ± 0.31^{b}
	ST	8.15 ± 0.19^{a}	9.19 ± 0.25^{a}	8.03 ± 0.70^{a}	6.41 ± 0.38^{b}
Chroma value (C*)	LD	21.46 ± 0.42^{b}	21.60 ± 0.26^{ab}	22.71 ± 0.35^{a}	21.23 ± 0.42^{b}
	ST	19.35 ± 0.32^{b}	21.73 ± 0.25^{a}	21.59 ± 0.55^{a}	19.54 ± 0.25^{b}
Hue angle (H°)	LD	29.21 ± 0.74^{a}	26.32 ± 0.85^{b}	26.33 ± 0.76^{b}	23.35 ± 0.72^{c}
	ST	24.95 ± 0.79^{a}	28.08 ± 0.92^{a}	21.70 ± 1.46^{ab}	19.12 ± 1.05^{b}
Color difference (ΔD)	LD	6.32 ± 0.68^{a}	4.59 ± 0.73^{ab}	6.45 ± 0.17^{a}	4.26 ± 0.52^{b}
	ST	6.76 ± 0.83^{a}	4.68 ± 0.43^{b}	4.47 ± 0.47^{b}	3.18 ± 0.50^{b}

$^{a, b, c}$ = The differences indicated by different letters on the same line are significant.

In the present study, the pH values were significantly different among breeds, but pH ranges in all breeds are acceptable when previous studies are evaluated[8,11,12,13]. Hair and Angora kids had higher pH than Kilis and Honamli breeds at 1 h and 24 h postmortem in LD and ST muscles. Moreover, pH drop from 1 h to 24 h postmortem in LD and ST muscles of Kilis kids higher than almost in the studied other goat breeds. The pH values at 24 h postmortem in LD muscle, in Kilis and Honamli breeds, were found similar to those founds by previous studies[11,12,13] in the different native goat breeds. Formation of severe stress associated with noise, transport, handling and slaughter may cause weight loss and a high muscle pH after slaughter in young animals[14]. Possibly, Hair and Angora breeds may be more sensitive than Kilis and Honamli breeds and highest pH value of they might also reflect differences in their responses to the stress of transport, handling, and lairage.

The water retain ability of fresh meat during application of external handling such as heating, grinding, cutting or pressing is an important indicator for meat quality[2]. Moreover, the water retain ability influence holding of vitamin and minerals that affect meat sensory properties such as juiciness and flavor as well as the volume of water retained[2,12]. Meats with poor water retention are easily and quickly drier and

Table 3 - Chemical composition of Longissimus-dorsi (LD) and Semitendinosus (ST) muscles from male kids born to Turkish indigenous goat breeds (% on dry matter).

Traits	Muscles	Kilis	Honamli	Hair	Angora
Dry matter	LD	22.56 ± 0.35^{b}	23.01 ± 0.12^{b}	24.89 ± 0.52^{a}	24.54 ± 0.47^{a}
	ST	22.94 ± 0.14^{b}	23.36 ± 0.29^{ab}	24.34 ± 0.44^{a}	23.79 ± 0.34^{ab}
Ash	LD	1.95 ± 0.13^{b}	2.91 ± 0.31^{a}	2.23 ± 0.10^{b}	1.80 ± 0.20^{b}
	ST	1.89 ± 0.06^{b}	3.39 ± 0.44^{a}	2.24 ± 0.11^{b}	2.06 ± 0.30^{b}
Protein	LD	21.99 ± 0.19	21.95 ± 0.30	23.39 ± 0.62	22.59 ± 0.43
	ST	21.87 ± 0.17	21.85 ± 0.21	22.46 ± 0.31	21.60 ± 0.39
IMF	LD	1.51 ± 0.25^{b}	1.37 ± 0.25^{b}	1.29 ± 0.10^{b}	2.12 ± 0.33^{a}
	ST	2.12 ± 0.14^{ab}	1.75 ± 0.19^{b}	1.63 ± 0.16^{b}	2.28 ± 0.12^{a}

$^{a, b, c}$ = The differences indicated by different letters on the same line are significant. IMF = intra-muscular fat.

weight loss increases during storage, transport and marketing. The water retain characteristics such as drip loss, cooking loss, frozen-thawing loss and water holding capacity in LD and ST muscles were clearly affected by breeds in the present study. Generally, the highest drip loss and cooking loss was determined in both muscles in Kilis and Hair breeds respectively, but lower water holding capacity was determined in Angora breed. Similarly, Madruga et al.[15] found significant differences among genotypes (Moxotó and Canindé) in terms of water holding capacity. However, our results for water holding capacity in both muscles were lower than results of Madruga et al.[15]. Differences among breeds in terms of cooking yield have also been reported by Schönfeldt et al.[16] and Dhanda et al.[7]. Johnson et al.[17], Dhanda et al.[7] and Sen et al.[18] have reported similar cooking losses, but Santos et al.[8] have reported lower cooking losses than the results of our study for goat meat. Differences in cooking losses among studies may be attributed to differences in age of kid, ultimate pH, cooking length, cooking temperatures and the muscles studied. Drip loss is an important indicator of meat quality[2]. Additionally, low drip loss in fresh meat indicates a high water holding capacity, greater juiciness, freshness, and a less-dry surface. The drip loss value is known to be negatively related to pH value in fresh meat but the magnitude of correlation differs between studies. Moreover, increased drip loss may be related to different factors, such as protein denaturation, sarcomere shortening and myosin denaturation[19]. Nayga et al.[20] reported that there were significant differences among goat breeds in terms of drip loss. In the present study, Kilis kids had relatively higher percentage of drip loss, while Angora kids had relatively lower percentage of drip loss values with high pH values. These observations are in agreement with the argument of Otto et al.[21], who reported high meat pH could actually be reduced drip loss. Lagerstedt et al.[22] reported that freezing decrease the meat quality and for this reason consumer prefer meat that has not been frozen. Thawing plays an important role in the processing of frozen meat due to the amount of leaking water during the thawing process, which is one of the criterias of the frozen meat quality[23]. In the present study, Angora kids had lower frozen-thawing loss with low water holding capacity. Therefore, it can be suggest that Angora meat more suitable for frozen during store compare to other Turkish native goat breed.

The evaluation of factors affecting meat tenderness is particularly important in goat meat because of its lower tenderness than sheep and beef[17]. Shear force values reported for goat meat vary considerably, depending on factors such as the treatment of the animals prior to slaughter and of the carcass post-mortem, the sampled muscle and method of sample preparation[24]. Hopkins et al.[25] indicated that shear force declines as intramuscular fat percentage increase. Moreover, previous studies reported that genotype had a significant effect on Warner-Bratzler shear force values in goat meat[8,12,26]. In the present study, tenderness were evaluated by the maximum shear force necessary to cut the meat perpendicular to the muscle fibers and it appears from present study that assessors identify differences in share force value among breeds and Angora kids had less tender meat obtained from LD and ST muscles than the other three breeds. This may be a consequence of their lower intra muscular fat contends in LD and ST muscles compared with the other breeds.

Muscle colour is extremely important in suckling kids' production whose carcasses should be pale or pink and one criterion by which consumers judge meat quality. The instrumental muscle colour values (L*, a* and b*) were significantly different among breeds at 1 h and 24 h postmortem and the meat colour from male kids born to Turkish indigenous goat breeds can be valued or classified as pale red. In the present study, L*, a* and b* colour values were affected by genotype, in agreement with Simela et al.[26] and Santos et al.[8], who studied indigenous South African and Portugal goat breeds, respectively. In the current study, the L* values in kids was lower than results of Sañudo et al.[13] in heavier animals, but a* and b* values were higher than results of same study. Redness reflects the presence of myoglobin and the availability of iron in muscles[13]. The Hair genotype displayed higher a* values (which vary in relation to haem pigment content) compare to other breeds in the present study. The main reason for this finding may probably the milk diet with low iron content received by the kids born to other breeds. These results were confirmed by results of previous studies the different level of haem pigment found among breeds[8]. Young offspring of small ruminants, especially non-weaned and fed with milk, have lightness meat. Consumers in Mediterranean countries associate meat from suckling kid as being tender, juicy, tasty and with a high price[11]. Angora genotype displayed lower L* and b* values compare to breeds, probably because these animals were fed substantially with milk, been weaned and slaughtered relatively early in the present study. Although L* can be positively correlated with myofibrilar structure and negatively with pH[27], it might have been more related to their evident paleness because of the limited amount of myoglobin[7]. Similarly, Şirin[28] reported that a positive correlation between type IIB muscle fiber number and L*, but negatively correlation with pH in LD muscle. Generally, in the both muscle of Kilis kids was higher in terms of L* values and lower in terms of a* value compare to the other kids' muscles in the present study. This could be related to its higher drip loss value and lower pH value at 24 h postmortem than those of the other kids. Similar to the findings in the present study, a significant effect of genotype on goat meat color has been reported by Madruga et al.[15] and Peña et al.[12]

Chroma (C*), which represents color saturation or purity, and hue angle (H°), defined as color wheel, with red-purple at angle 0° and 360°, yellow at 90°, bluish-green at 180° and blue at 270°, were calculated from a* and b* parameters in the present study. Marichal et al.[27] reported that muscle color characteristics and carcass weight have significant effect on muscle C* and H° values. Moreover, Teixeira et al.[29] suggested that a decrease in H° value and an increase in C* value are related with a carcass weight increase. In the present study carcass weight of kids born to Hair, Angora, Kilis and Honamli breeds were similar (data not shown), but C* and H° values was, between goat breeds, higher in the both muscles from Angora, with Kilis in terms of C* value. Although C* value is influenced less by the chemical state (oxidative process) of the myoglobin than H° value of meat[12], the difference in Chroma (C*) and hue angle values of the muscles among breeds can be attributed to enzymatic reducing system or the sensitivity to oxidative change. Unfortunately, these parameters were not investigated in the present study.

The chemical composition of carcass meat is one of the best predictors of nutrient component and meat quality[1]. Marichal et al.[27] reported that moisture ranged from 77.2 to 78.5%, total protein from 18.1 to 20.7%, intramuscular fat from 0.9 to 1.3% and ash from 1.1 to 1.2% of meat from kids with different live weights. In the present study, the dry matter (22.56%-24.89%), total protein (21.60%-23.39%), intramuscular fat (1.29%-2.28%), and ash (1.80%-3.39%) contents of LD and ST muscle samples of male kids born to Turkish indigenous goat breeds were considered as acceptable for fresh kids meat on sale. Meat chemical analysis revealed that breed had significant effect on dry matter, intramuscular fat and ash content, in the proximate composition analysis of kids born to four Turkish native goat breeds. Oman et al.[30] reported that Angora goats had lighter live and hot carcass weights than other studied goat breeds and crossbreeds (Spanish, Boer × Spanish and Spanish × Angora) when slaughtered at a given age. Moreover, in the study of Oman et al.[30] primal carcasses cuts of Angora breeds were always the fattest or among the fattest compare to other studied goat breeds and crossbreeds. In the current study intramuscular fat content in LD and ST muscles of Angora kids were higher than the studied other goat breeds. These observations are in agreement with the argument of Oman et al.[30]. Wood[31] reported that 2 to 3% of intramuscular fat is needed to ensure the organoleptic qualities of meat; therefore, Angora kids potentially presented a better intramuscular fat distribution. Dry matter percentages reported by Marichal et al.[27] were similar with our results for kids slaughtered at same live weights. Additionally, total protein and ash percentages in the present study were similar to reports of Marichal et al.[27] and Kesava et al.[32] for goat kid meat.

In conclusion, the results obtained from present study indicate that there is a breed effect on meat quality characteristics and chemical composition at weaning. Differences among Turkish indigenous goat breeds in terms of meat quality characteristics may help to promote alternative kid meat or meat products for the consumers. The obtained results are key elements in functional meat production as a key input. Additionally, meat quality parameters of all breeds were commercially acceptable for consumer and market preferences especially for Mediterranean regions.

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Parasitological investigation in an organic dairy donkey farm

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SUMMARY

A parasitological survey was carried out in an organic dairy donkey farm. Individual fecal, skin and fur samples were collected from 29 donkeys (14 jennies, 2 jacks, 9 fillies and 4 foals) and examined for the search of ecto- and endoparasites. Blood samples were also collected from jennies and fillies and examined for the presence of IgG antibodies to *Toxoplasma gondii*. All donkeys were found infected by intestinal strongyles (100%), with a fecal egg count ranging from 50 to 2400 eggs per gram of feces. Moreover, the following endoparasite species were also identified: *Oxyuris equi* 37.9%, *Parascaris equorum* 31%, *Dicrocoelium dendriticum* 14.3%, *Fasciola hepatica* 14.3%, Anoplocefalidae cestodes (*Anoplocephala perfoliata*, *Anoplocephala magna* and *Anoplocephaloides mamillana*) 10.3%, and *Strongyloides westeri* 6.8%. A single jenny (4.3%) was found positive to *T. gondii* at serological analysis. The louse species *Haematopinus asini* was detected in 27.6% animals. Multiple parasite infections were found in the 76% of examined donkeys. This is the first parasitological investigation in an organic dairy donkey farm worldwide. Results showed that pathogenic helminths and *H. asini* are prevalent in the examined donkeys, suggesting the need for effective parasite control measures.

KEY WORDS

Dairy donkeys, ectoparasites, endoparasites, central Italy, prevalence, organic farm.

INTRODUCTION

Recently, the interest in donkey farming has increased in several European countries, mainly due to the recent popularity gained by donkey milk use for the cosmetic industry or for human consumption, and the use of donkeys for social and recreational activities[1,2]. Consequently, the interest in the diseases of donkeys has also increased[3,4]. Amongst donkey pathogens, parasite infections are frequently observed and may negatively affect the health, the productive and reproductive performances of infected animals[3,4,5]. Prevalent donkey endoparasites include the intestinal roundworm species *Parascaris equorum* and intestinal strongyles, that in infected donkeys may be the cause of weight loss and reduced growth and productions, decline in general conditions, intestinal obstruction, colic and diarrhea[5]. Among donkey endoparasites, the tickborne apicomplexan protozoa *Babesia caballi* and *Theileria equi* are prevalent in donkeys in Italy and are considered a possible cause of poor work performance in asymptomatic donkeys[6,7]. Some endoparasites, especially *Toxoplasma gondii*, are zoonotic species and *T. gondii* infected donkeys are considered a potential source for human infections through the consumption of donkey milk and meat products[8,9].

Among ectoparasites, lice and other arthropods are frequently observed in donkeys and are often responsible for anemia, pruritus, skin lesions and debilitation, mainly in younger animals[10].

The diffusion, intensity and species composition of parasite infections may be highly variable in donkeys, depending on several factors, such as farming system, management practices and geographical area[5,11]. Although parasitic infections are considered extremely important in donkeys, few recent studies concern with parasite infections in donkeys in Europe[3,4,8,10,12,13]. Moreover, data on parasitic infections in organic donkey farms are completely absent.

In this study, a parasitological investigation was carried out in an organic dairy donkey farm aimed at evaluating the prevalence and species composition of ecto- and endoparasite infections.

MATERIALS AND METHODS

Animals

Twenty-nine donkeys of different breed (Romagnola, Amiatina and cross-bred animals), gender and age, reared outdoor in a semi-extensive system in an organic dairy donkey farm according to the EU regulations 834/2007 and 889/2008, and located in a natural reserve of central Italy (Arezzo, Italy; 43° 39' 3.3" N, 12° 10' 10.51" E), were examined. Except for five foals, all animals reared in the farm were included in the study. The reserve is mainly a vast wooded area (1540 hectares) covered with forests for about 86% of its surface,

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rich in waterways and placed at an altitude ranging from about 520 to 1453 meters above sea level. Several wild animal species live in this natural reserve, including deer.
The farm covers about 280 hectares and includes stables and grassy or bushy pastures and mowing grassland delimited by wooden and electric fences. The donkeys had free access to pastures during the experimental period. The main farm production is pasteurized donkey milk for human consumption according to E.U. regulation 853/2004, and for the cosmetic industry. Moreover, donkeys are also used for educational and recreational activities. In the farm, donkeys are fed ad libitum with an in farm-produced mixed-grass hay. About 2.5-5 kg of concentrate/day/donkey are also administered, depending on the age and the productive and reproductive needs of animals.
Examined animals included 14 jennies (8-15 years old), 2 jacks (5 years old), 9 fillies (3-4 years old) and 4 foals (10-12 months old). No antiparasitic treatments had been performed in the farm in the 12 months prior the study.
Approval for this study was obtained from the Ethical Committee on Animal Experimentation of the University of Pisa. In addition, authors declare that the work was carried out in compliance with relevant European guidelines regarding ethical use of animals and in adherence to a high standard of veterinary care.

Sampling

Individual fecal samples were collected from the rectal ampoule of all examined animals and examined for the detection of endoparasites. Moreover, the perianal region of each animal was dabbed with strips of transparent adhesive tape for the search of oxyurid eggs, that were adhered on a microscope slide (scotch test). Blood samples were also collected from jennies and fillies, via venipuncture of a jugular into tubes without anticoagulant. Sera were separated by centrifugation for 10 min at 1000 × g and stored at -20°C until examination. Furthermore, individual hair and skin samples were collected both by skin scrapings and by using the scotch-tape test from animals showing skin lesions (alopecia, erythema, scabs), to detect ectoparasite arthropods. All samples were individually labeled, immediately refrigerated at 4°C after the collection, and processed within 12-24 hours. When visible to the naked eye, ectoparasites were directly collected with a needle from the infested animals and placed in test tubes containing 80% ethyl alcohol. Except for blood samples that were collected once, sampling was performed twice, in summer (July) and autumn (November).

Parasitological analysis

For the search of endoparasites, faecal samples were qualitatively examined with a sedimentation/flotation technique[14] using a saturated ZnCl solution (specific gravity 1.56). In addition, a McMaster technique with a sensitivity of 50 eggs per gram of faeces (EPG) was also performed for counting nematode and cestode eggs, by using saturated NaCl as flotation solution (specific gravity 1.2). The Baermann technique was used for the detection of *Dictyocaulus arnfieldi* larvae in faecal samples. Moreover, a commercial rapid immunoassay (RIDA QUICK®) was used to detect *Giardia duodenalis* and *Cryptosporidium* spp. faecal antigens. For the identification of intestinal strongyles such as Strongylinae or Cyathostominae, coprocultures were also made with pooled faecal samples positive for these parasites, placed in an incubator at 27°C for seven days and larvae were recovered using the Baermann technique[15]. Larvae (about 100 larvae) were microscopically examined and identified according to morphological keys as previously reported[16]. Anti-*Toxoplasma gondii* IgG antibodies were detected from sera of all adult females and fillies using a modified agglutination test (MAT), as previously described[17].
Skin and hair samples collected by scotch-tape and skin scrapings were microscopically evaluated for the presence of ectoparasite arthropods as fresh samples or after exposure to 10% NaOH at 30°C for about 30 min, to dissolve hairs and epidermal scales. Moreover, collected ectoparasites were mounted in Hoyer medium, observed under an optical microscope and identified at species level using morphological keys and descriptions given by Ferris[18].

Statistical analysis

Data were analyzed using R software (R Core Team 2015). The prevalence of identified parasites was estimated as the number of positive animals/total number of examined animals. Prevalence of each identified parasite in the two different seasons (summer and autumn), were compared by using the Student's T test. The significance level was set at $P< 0.05$.

RESULTS

All examined animals were found infected by at least a single parasite species (Table 1, Figure 1). Multiple parasite infections were found in about 76% (22/29) of examined donkeys (Tables 1 and 2).
Among faecal parasites, all animals (29/29, 100%) were found infected by intestinal strongyles. Coprocultures revealed a high prevalence of cyathostomins (>90%) in the examined farm.
At quantitative analysis, the EPG number of these parasites was found highly variable, ranging from 50 to 2400 EPG. Considering both seasons, 23 out of 29 animals showed a fecal count ≥200 EPG, and in 7 donkeys the fecal egg count was higher than 1000 EPG. In adult donkeys, the average EPG number of intestinal strongyles was similar in the two seasons, i.e. 493 ± 530 EPG in summer and 482 ± 458 EPG in autumn (Table 1).
The ascarid species *P. equorum* showed an overall prevalence of 31% (9/29), but its prevalence was highly variable in the different age-groups of animals. In fact, the prevalence of *P. equorum* was 31.25% (5/16) in adult donkeys, 22.2% (2/9) in fillies and 75% (3/4) in foals (Table 2).
The pinworm *Oxyuris equi* showed a prevalence of 37.9% (11/29). However, this nematode was identified only in adult animals, including the adult males (2/2, 100%) and 9/14 jennies (64.3%).
Strongiloydes westeri showed an overall prevalence of 6.8% (2/29) and was identified only in 2 fillies.
Anopocephafalidae cestodes showed an overall prevalence of 10.3% (3/29) and were identified only in jennies and fillies. *Anoplocephala perfoliata* was the most frequent species (3/29, 10.3%), while *Anoplocephaloides* (*Paranoplocephala*) *mamillana* (2/29, 6.8%) and *Anoplocephala magna* (1/29, 3.4%) were less frequent. In summer, these parasites showed a lo-

wer prevalence (6.9%) and a lower mean EPG number (50 EPG) than in autumn (prevalence 10.3%, 200 EPG in average), but these differences were not statistically significant.

Among trematodes, *Fasciola hepatica* and *Dicrocoelium dentriticum* were identified and both these parasites showed an overall prevalence of 13.8% (4/29). However, *F. hepatica* was

Table 1 - Ecto- and endoparasites identified in animals of an organic dairy donkey farm from central Italy.

Donkey	Summer	Autumn
JENNIES		
1	50 EPG Gastrointestinal Strongyles	Deceased
2	200 EPG Gastrointestinal Strongyles	100 EPG Gastrointestinal Strongyles
3	250 EPG Gastrointestinal Strongyles	200 EPG Gastrointestinal Strongyles *Haematopinus asini*
4	300 EPG Gastrointestinal Strongyles	200 EPG Gastrointestinal Strongyles
5	50 EPG Gastrointestinal Strongyles *Oxyuris equi** *Parascaris equorum** *Fasciola hepatica**	300 EPG Gastrointestinal Strongyles
6	1050 EPG Gastrointestinal Strongyles *Oxyuris equi** *Parascaris equorum** *Fasciola hepatica**	200 EPG Gastrointestinal Strongyles
7	2400 EPG Gastrointestinal Strongyles *Oxyuris equi** *Parascaris equorum** *Fasciola hepatica**	100 EPG Gastrointestinal Strongyles
8	350 EPG Gastrointestinal Strongyles *Oxyuris equi** *Parascaris equorum** *Fasciola hepatica**	100 EPG Gastrointestinal Strongyles
9	500 EPG Gastrointestinal Strongyles	300 EPG Gastrointestinal Strongyles
10	300 EPG Gastrointestinal Strongyles 50 EPG *Anoplocephaloides mamillana* *Anoplocephala perfoliata**	500 EPG Gastrointestinal Strongyles *Anoplocephala perfoliata**
11**	250 EPG Gastrointestinal Strongyles	900 EPG Gastrointestinal Strongyles 200 EPG *Parascaris equorum* *Haematopinus asini*
12	400 EPG Gastrointestinal Strongyles	200 EPG Gastrointestinal Strongyles *Oxyuris equi** *Haematopinus asini*
13	600 EPG Gastrointestinal Strongyles	600 EPG Gastrointestinal Strongyles *Oxyuris equi** *Toxoplasma gondii* *Haematopinus asini*
14	1750 EPG Gastrointestinal Strongyles *Oxyuris equi** *Dicrocoelium dentriticum** *Haematopinus asini*	100 EPG Gastrointestinal Strongyles 100 EPG *Parascaris equorum* *Oxyuris equi** *Haematopinus asini*

Donkey	Summer	Autumn
JACKS		
1	600 EPG Gastrointestinal Strongyles	1100 EPG Gastrointestinal Strongyles 50 EPG *Parascaris equorum* *Oxyuris equi**
2	750 EPG Gastrointestinal Strongyles	2100 EPG Gastrointestinal Strongyles *Oxyuris equi**
FILLIES		
1	700 EPG Gastrointestinal Strongyles *Strongyloides westeri** *Dicrocoelium dentriticum**	100 EPG Gastrointestinal Strongyles *Haematopinus asini*
2	100 EPG Gastrointestinal Strongyles *Dicrocoelium dentriticum**	900 EPG Gastrointestinal Strongyles
3	150 EPG Gastrointestinal Strongyles *Dicrocoelium dentriticum**	100 EPG Gastrointestinal Strongyles
4	1100 EPG Gastrointestinal Strongyles 50 EPG *Parascaris equorum* *Haematopinus asini*	200 EPG Gastrointestinal Strongyles *Haematopinus asini*
5	200 EPG Gastrointestinal Strongyles 50 EPG *Strongyloides westeri*	200 EPG Gastrointestinal Strongyles
6	250 EPG Gastrointestinal Strongyles	800 EPG Gastrointestinal Strongyles
7	50 EPG Gastrointestinal Strongyles 50 EPG *Parascaris equorum* 50 EPG *Anoplocephala perfoliata*	300 EPG Gastrointestinal Strongyles 100 EPG *Parascaris equorum* 150 EPG / 50 EPG *Anoplocephala perfoliata*
8	300 EPG Gastrointestinal Strongyles	800 EPG Gastrointestinal Strongyles
9	250 EPG Gastrointestinal Strongyles	300 EPG Gastrointestinal Strongyles 100 EPG *Anoplocephaloides mamillana* 100 EPG *Anoplocephala magna* 100 EPG *Anoplocephala perfoliata*
FOALS		
1	100 EPG Gastrointestinal Strongyles 550 EPG *Parascaris equorum*	200 EPG Gastrointestinal Strongyles 450 EPG *Parascaris equorum* *Haematopinus asini*
2	850 EPG Gastrointestinal Strongyles	1100 EPG Gastrointestinal Strongyles 100 EPG *Parascaris equorum*
3	200 EPG Gastrointestinal Strongyles 300 EPG *Parascaris equorum*	700 EPG Gastrointestinal Strongyles
4	250 EPG Gastrointestinal Strongyles	800 EPG Gastrointestinal Strongyles

* = Positivity detected only at the sedimentation/flotation technique performed on faecal samples. ** = Jeanny tested positive to *T. gondii* antibodies at serological analysis.

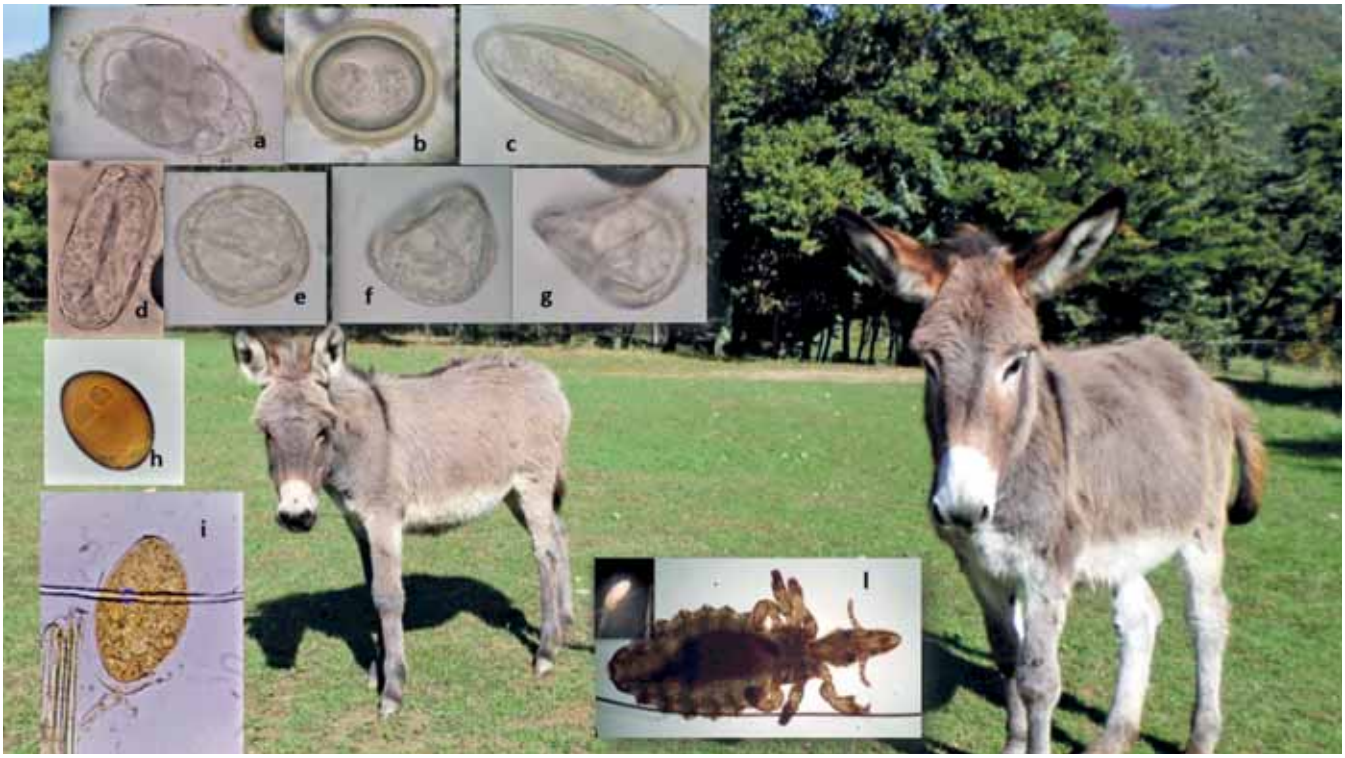

Figure 1 - Endo- and ectoparasites identified in an organic farm from central Italy: **a.** intestinal strongyle egg (200x); **b.** *Parascaris equorum* egg (200x); **c.** *Oxyuris equi* egg (200x); **d.** *Strongyloides westeri* egg (400x); **e.** *Anoplocephala magna* egg (200x); **f.** *Anoplocephala perfoliata* egg (200x); **g.** *Anoplocephaloides mamillana* egg (200x); **h.** *Dicrocoelium dendriticum* egg (400x); **i.** *Fasciola hepatica* egg (100x); **l.** *Haematopinus asini* adult and egg (40x).

identified only among jennies (28.6%, 4/14), while a single jenny (1/14, 7.1%) and 3/9 fillies (33.3%) were found positive for *D. dentriticum*. Moreover, these two trematode species were found significantly more prevalent in autumn than in summer ($p< 0.05$).

All examined donkeys scored negative for intestinal protozoa, i.e. *Cryptosporidium*, *Giardia duodenalis* and *Eimeria leuckarti*. Similarly, no animal was found positive for the respiratory nematode *Dictyocaulus arnfieldi*.

At serological analysis, a single jenny tested positive to *T. gondii* antibodies (1/23, 4.3%) with a low titre (1:40).

Regarding ectoparasites (Table 1), adults and eggs of the blood suckling louse species *Haematopinus asini* were identified at microscopical examination in 8/29 animals (27.6%), including jennies, fillies and foals. Most of the infested animals showed skin lesions, mainly characterized by crusty areas. Moreover, *H. asini* showed a significant lower prevalence ($p< 0.05$) in summer than in autumn.

Table 2 - Prevalence (%) of faecal parasites identified in an organic dairy donkey herd in central Italy according to the different age and gender. Examined animals included 14 mares (of 8-15 years in age), 2 adult males (of about 5 years in age), 9 fillies (of 3-4 years in age) and 4 foals (of 10-12 months in age).

	Jennies	Jacks	Fillies	Foals
Gastrointestinal strongyles	100%	100%	100%	100%
Parascaris equorum	35.7%	50%	22.2%	75%
Oxyuris equi	42.8%	100%	–	–
Strongiloydes westeri	–	–	11%	–
Dicrocoelium dentriticum	7.1%	–	33.3%	–
Fasciola hepatica	28.5%	–	–	–
Anoplocephaloides mamillana	7.1%	–	11.1%	–
Anoplocephala magna	–	–	11.1%	–
Anoplocephala perfoliata	7.1%	–	22.2%	–

DISCUSSION

Several parasite species are included among the main pathogens of donkeys[19]. Nevertheless, in Europe few data are available about the distribution and variability of donkey intestinal parasite infections[3,12]. This is especially true in the case of organic donkeys reared to produce milk for human consumption. Nevertheless, in organic farms the knowledge of prevalence, intensity, species composition and distribution of parasites among different age-groups of animals, is considered essential to perform effective and sustainable control measures to combat and manage parasite infections[20].

Among endoparasites, intestinal strongyles were identified in all donkeys examined in this study. These finding confirm the high prevalence of intestinal strongyles previously observed in donkeys worldwide[5], Italy included[12]. The high prevalence of intestinal strongyles in sampled animals may depend mostly on the lack of anthelmintic treatments and possible high pasture contamination. Intestinal strongyles are included among the most important donkey parasites and may damage animals both in larval and adult stage[5]. It is a group of nematodes with a cosmopolitan distribution that in adult stage localize in the large intestine of equids. Intestinal strongyle species infecting donkeys belong mostly to the subfamilies Strongylinae and Cyathostominae, also known as large and small strongyles, respectively[5]. As observed in this study, a higher prevalence of cyathostomins are generally ob-

served in European donkey farms[5,21]. Cyathostomin infections tend to be higher in young donkeys, but adult animals are often infected and may contribute to pasture contamination[5]. The faecal egg count of intestinal strongyles observed in donkeys is generally higher than in horses[22]. Nevertheless, most of donkeys found positive in this study showed a fecal count of intestinal strongyle eggs ranging from 500 to 1000 EPG or higher, at least in one of the two examined seasons.

The ascarid species *P. equorum* was a further species found prevalent (31%) in this study and both jennies and younger animals were found infected. These results confirm some previous observations on *P. equorum* infections in adult donkeys and the important role they may play in pasture contamination[5]. Nevertheless, in foals the prevalence of this nematode species was higher (75%) than in jennies and fillies.

The pinworm *O. equi* is a worldwide-diffused nematode whose localization site is the large intestine of equids and it is considered as a nuisance or irritant, low pathogenic parasite[23]. In this study, this nematode showed an overall prevalence of 37.9%, but it was identified only in adult animals, with a prevalence of 42.8% in jennies and 100% in males. These prevalence rates are higher than what reported in previous studies (about 1-4%)[24,25], probably because in the present survey both flotation test and scotch test of the perineal skin were used in parallel for the detection of this nematode.

About *S. westeri*, the prevalence observed in this study for this nematode species was low (6.8%). In equids, this parasite is considered widespread mostly among very young animals[26], while in the present study it was identified only among fillies.

In previous studies, *D. arnfieldi* has been reported as a nematode species occurring in donkeys with a prevalence ranging from about 3.6% to about 19%[5,12]. However, in the present survey none of the examined faecal samples was found positive for this respiratory nematode.

Among trematodes, *F. hepatica* showed an overall prevalence of 13.8%, but it was identified only in jennies (28.5%). *F. hepatica* is a common liver parasite of ruminants, but it can infect also other animals, including equids and humans[22,27]. In European donkeys, this trematode has been mainly found at necropsy examination of deceased animals[3]. It is generally accepted that clinical fascioliasis in equids is rare, but in heavily contaminated areas animals grazing with ruminants may suffer from sub-acute or chronic diseases[25].

D. dentriticum is a further liver fluke species that typically infects ruminants. In this study *D. dentriticum* showed a higher prevalence (overall 13.7%, 7.1% in jennies and 33.3% in fillies) when compared to that previously reported in donkeys (0.9-12.8%)[28]. As for *F. hepatica* infection, the contamination of pastures by infected wild ruminants may represent the main reason for the high prevalence of *D. dentriticum* in examined donkeys.

In this study, anopocephalid cestodes were detected with a higher prevalence (10.3%) compared to that reported in previous studies[24,29]. *A. perfoliata* was the most prevalent species (10.3%), as evidenced in previous studies[5], while *A. mamillana* and *A. magna* were less frequent. Although these cestodes are a recognised cause of colic in horses, their clinical effects in donkeys are not yet completely known[5].

The protozoans *G. duodenalis, Cryptosporidium* spp. and *Eimeria leuckarti* have been reported with variable prevalence in donkeys worldwide[30-32]. However, in the present study no positivity to these faecal protozoans was recorded.

T. gondii showed 4.3% seroprevalence in this study. This value is in line with what has been observed in a study carried out on donkeys in Italy[13]. Even though only one jenny showed a positive result to *T. gondii* antibodies, this finding may indicate a potential risk of human infection. Indeed, seropositive lactating jennies, also those with a low serological titre (1:20, 1:40), can be positive to *T. gondii* DNA in milk and blood as demonstrated in a previous study[8]. Thus, the consumption of infected raw milk and other raw donkey products can be a further source of infection to humans, mainly to babies and children where donkey milk is often administered in case of cow milk allergy due to its similarity in composition to woman's milk[2].

Among ectoparasites, the blood suckling louse *H. asini*, previously reported in donkeys also in Italy[10], was the only species identified in this study. In infested donkeys, *H. asini* is considered often responsible for anemia, pruritus and skin lesions, reduction of body conditions and weakness[10]. Subclinical infestations caused by a low number of parasites are also frequently observed and may contribute to the diffusion of *H. asini* among donkeys[10].

CONCLUSIONS

Our results showed that intestinal helminths and the louse species *H. asini* are prevalent in the donkey organic farm here examined. Among faecal parasites, mainly infections caused by intestinal strongyles, but also those caused by ascarids, pinworms and anoplocephalid cestodes, were found prevalent. The identification of zoonotic fluke species and of serological positivity to *T. gondii* is also noteworthy.

The high frequency of multiple infections and the pathogenicity and intensity of some identified parasite species may be responsible for important reductions in productive and reproductive performances and, potentially, also for overt disease in examined donkeys[5].

In this farm, it is therefore advisable the use of effective control measures. However, in organic dairy donkeys reared to produce milk for human consumption, the control of parasites should mainly be based on alternative methods to the use of drugs. Various alternative approaches for parasite control have been extensively studied in equine husbandry, although mainly in horses. Among these different management-based nematode control methods are included, such as the removal of faeces from pastures, rotational grazing and the administration of food supplements[33-35]. The use of nematophagous fungi[35] and of plant-derived compounds[33,36], has been also recommended for the control of nematodes and lice. Therefore, some or all these control methods could be used in the organic dairy farm examined in the present study. Effective methods to avoid the access of wild animals in donkey grazing areas may be also helpful for the control of liver fluke infections.

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Evaluation of two commercial dietary enzyme products in broiler chicken fed reduced energy diets based on corn and soybean meal

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SUMMARY

This study evaluated the effects of two commercial enzyme products incorporated into reduced energy diets on growth performance, mortality and litter quality of broiler chicken. A total of 1620 7-d-old male Arbor Acres broiler chicks were randomly divided into 3 groups of 540 chicks each (60 chicks x 9 replications) and assigned to one of the following treatments: (1) corn-soybean meal based diet as control (C), (2) C with a 60 Kcal/kg AME reduction supplemented with NSP-degrading enzymes (Rovabio Excel) at 0.05 g/kg (D1), and (3) C with a 120 Kcal/kg AME reduction supplemented with multi-enzyme preparation (Natuzyme) at 0.1 g/kg (D2). There were 4 dietary phases: starter (d1-d7), grower (d8-d21), finisher 1 (d22-d28), and finisher 2 (d29-d37). The experimental period was d7-d37. Live body weight (LBW), daily weight gain (DWG), daily feed intake (FI), feed conversion ratio (FCR) and mortality rate were measured by production phase and for the whole rearing period (d1-d37). Production index (PI) and litter quality were also measured. No difference was seen between diets C and D1 during any stage or overall rearing period, showing that Rovabio Excel supplementation with reduced energy diet formulation completely compensated for the reduced energy amount. Natuzyme partially restored broiler performance equal to standard diet formulation, except for LBW which was lower ($p<0.05$) during all phases, DWG which was lower ($p<0.05$) on days 29-37, and FCR which was higher ($p<0.05$) on days 22-28 in Natuzyme supplemented group. Natuzyme supplemented diet produced lighter broilers with higher FCR at d 37 ($p<0.05$). Mortality and litter quality were not affected by enzyme supplementation. Rovabio Excel supplementation reduced the cost per kilogramme of live body weight. In conclusion, a 60 Kcal/kg AME reduced energy diet supplemented with Rovabio Excel had equivalent performance to a standard diet and provided the best economic result. This approach can be used to reduce the amount of primary ingredients needed to formulate poultry diets namely corn which is exclusively imported and consequentlty to decrease production costs.

KEY WORDS

Broilers performance; energy level; litter quality; Natuzyme; Rovabio Excel.

INTRODUCTION

In Tunisia, broiler chicken feed is based primarily on corn and soybean meal, which supplies the majority of energy and protein in the diet. Utilization of the nutrients contained in both feed ingredients by broilers is generally considered to be high. Nevertheless, it has been shown that about 400-450 Kcal of energy per kg of diet is not digested when birds are fed a typical corn-soya diet[1]. These feed ingredients are exclusively imported into Tunisia. In addition, the high demand for corn and soybean for human consumption and biofuel production has led to a surge in their prices world wide, consequently increasing feed cost which represents between 60 and 80% of the production cost of broiler chicken. Thus, the application of nutritional approaches that optimize feed utilization is needed to increase broiler efficiency and reduce production costs.

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Over the last two decades, several exogenous enzymes have become readily available and commonly used in poultry diets to improve feed utilization and performance. These exogenous enzymes are used either to supplement a lack of specific endogenous enzymes for degrading certain nutrients or to hydrolyse anti-nutritional compounds in feed ingredients. For example, dietary non-starch polysaccharides (NSP)-degrading enzymes and phytase have been used to reduce the negative effects of NSP[2;3;4] and enhance dietary phytate utilization[5;6], respectively. More recently, multiple-enzyme preparations with broad spectrum activity (e.g. NSPase activity, amylase activity, protease activity and phytase activity) are also being developed and used commercially. Their application has been shown to result in additive or synergistic effects on nutrient utilization and animal performance[7;8]. In some cases, these enzyme cocktails have been shown to improve nutrient utilization in poultry diets better than single enzyme products[9] but bird responses to enzyme supplementation are variable. Several factors contribute to these inconsistencies, principally enzyme type and concentration, diet, and bird factors such as genetics and the composition of the gastrointestinal microbiome. Among dietary factors, the

nutrient density of the diet is particularly important. Exogenous enzymes can be beneficial in diets containing low-digestible feedstuffs and a marginal nutrient density[9] or with diets containing high-digestible feedstuffs. Responses to exogenous enzymes are predicted to be minimal if there is a surplus of nutrients (energy, amino acids, phosphorus) in the diet. Accordingly, the use of some dietary enzymes has been suggested as a tool that can improve nutrient utilization in diets formulated with reduced available metabolic energy, crude protein or amino acids, available phosphorus or calcium[1;9;10;12]. This approach could be used to reduce the amount of primary ingredients such as corn and soybean meal needed to formulate poultry diets and consequentlty to decrease production costs. The purpose of this study was therefore to compare standard diet formulation to reduced energy diet supplemented with one of two commercial enzyme products, with respect to performance parameters, mortality and litter quality.

MATERIALS AND METHODS

Enzymes products used

The two commercial enzymes products tested in this study are as follows: (1) **Rovabio Excel** (ADISSEO, Alpharetta GA, USA) which is a combination of non-starch polysaccharides (NSP) degrading enzymes produced by the non-genetically modified fungus *Penicillum funiculosum*. The main enzymes are xylanase (Endo - 1,4 - β - xylanase, 30,000 unit/kg) and β-glucanase (Endo - 1,3 (4) - β - glucanase, 25,000 unit/kg). This product hydrolyzes pentosans and β glucans in plant raw materials; and (2) **Natuzyme** (Bioproton Pty Ltd., Sunnybank, Australia) is composed of xylanase (10,000,000 unit/kg), cellulase (5,000,000 unit/kg), β-glucanase (1,000,000 unit/kg), pectinase (140,000 unit/kg) from *Trichoderma reesei* and *Trichoderma longibrachiatum*. It also contains protease (6,000,000 unit/kg) and phytase (500,000 unit/kg) from *Aspergillus niger*, and α-amylase (1,800,000 unit/kg) from *Bacillus subtilis*. Each of these two enzyme products are claimed to enable better nutrient utilisation from feed, resulting in better growth performance and lower total costs.

Experimental feed preparation

A conventional feed (control diet; C) for broiler chicken based on corn and soybean-meal and two reduced energy diets supplemented with enzyme preparations (D1 and D2) were individually prepared for each production phase: grower phase (d8-d21), finisher 1 phase (d22-d28), and finisher 2 phase (d29-d37). D1 corresponds to C with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg. D2 correspond to C with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1 g/kg. Each enzyme preparation was incorporated into the assigned diet at the concentration recommended by the manufacturer. Enzymes were heat stable and able to with stand pelletization temperature up to 90°C for 90 s. For each enzyme product, the amount of reduced energy corresponds to the amount of improvement provided by its inclusion as compared to a standard corn-soybean meal based diet (without energy reduction) as claimed by the producer. To verify the producers' affirmation, the two reduced energy diets supplemented with enzyme preparations were individually compared to the standard diet. Since we were not interested to assess the amount of improvement provided by the dietary enzyme supplementation as compared to low energy diet without enzyme, no negative control diet was included in this study.

The composition and nutrient calculated content of the four diets for each production phase are given in Table 1.

Experimental design

One thousand six hundred and twenty (1,620) 1-day-old male Arbor Acres broiler chicks from a local commercial hatchery (Couvoir SAVINORD, Jendouba, Tunisia) were used to evaluate the effects of the dietary incorporation of enzyme preparations on the growth performance and litter quality. Upon their arrival, chicks were individually weighed and randomly distributed into 27 floor pens (2 m × 2 m i.e. 15 chicks per m^2) in a completely randomized design (3 treatments × 9 replications, each replication included 60 chicks). The 3 experimental groups were designed as follows: (1) a control group was fed a standard diet unsupplemented with enzymes (C), and (2) two groups were each fed one of two energy deficient diets supplemented with enzymes (D1or D2) described above.

During the first week of age (d1-d7), chicks in the control group received a standard starter diet, while those in D1 and D2 group received starter diets with reduced levels of energy corresponding to the same amount of reduced energy to be applied during the following production phases but without enzyme supplementation since exogenous enzyme supplementation during the starter period could have detrimental impact on chicks' health. Thus, the distribution of enzyme-supplemented diets started from the 8th day of age.

In all groups, feed and water were offered *ad libitum*. The lighting schedule was 23h light/1h darkness. Ambient temperature was equal to 33°C during the first week and it was subsequently reduced by 4°C each week. Wood shavings were used as litter material and spread in each pen to a thickness of 6 cm. The experimental protocol was approved by the Official Animal Care and Use Committee of the College of Agriculture of Mateur - University of Carthage before the initiation of research and followed the Tunisian guidelines approved by the committee on care, handling, and sampling of the animals.

Performance monitoring

During the experimental period (d8-d37), feed intake per pen and individual body weight were recorded for each production phase to calculate daily body weight gain (g/bird/day) and feed conversion rate (**FCR**). Mortality was daily monitored. Production index (**PI**) was also calculated as follows: [liveability (%) × final live body weight (kg) / growing period (37 days) × FCR] × 100.

Litter quality

Litter quality was assessed on d21, d28 and d37. Each pen was divided into 2 halves and litter quality was scored for each half of the pen and averaged. Litter quality scores were taken visually, and ranged from 1 to 4, with 1 being extremely dry and no caked litter and 4 being total pen coverage of caked litter. Three independent observers were involved in the litter scoring process to obtain the average value.

Table 1 - Composition and nutrient calculated content of experimental diets.

Item	Grower (8-21d)			Finisher 1 (22-28d)			Finisher 2 (29-37d)		
	C[1]	D1[2]	D2[3]	C	D1	D2	C	D1	D2
INGREDIENT (%)									
Corn	44.27	42.12	40.82	40.93	39.07	38.21	37.39	35.99	34.58
Soybean meal	23.21	22.47	23.75	19.53	19.29	19.29	17.42	19.10	18.93
Wheat	20.11	23.22	23.22	26.91	29.24	30.00	32.85	32.75	34.50
Vegetable oil	07.50	07.35	07.36	08.00	07.80	07.96	08.10	07.95	07.80
Premix[4]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	0.74	0.68	0.68	0.52	0.50	0.42	0.20	0.16	0.14
Limestone	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
NaCl	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
DL-Met	0.30	0.30	0.30	0.26	0.26	0.26	0.23	0.23	0.23
L-Lys HCl	0.26	0.26	0.26	0.26	0.24	0.26	0.23	0.23	0.23
L-threonine	0.13	0.11	0.12	0.11	0.11	0.11	0.10	0.10	0.10
Rovabio Excel	–	0.005	–	–	0.005	–	–	0.005	–
Natuzyme	–	–	0.01	–	–	0.01	–	–	0.01
NUTRIENT									
AME (Kcal/kg)	3050	2990	2930	3180	3120	3060	3240	3180	3120
Crude protein (%)	20.00	20.00	20.00	18.20	18.20	18.20	18.00	18.00	18.00
Ca (%)	0.95	0.95	0.95	0.90	0.90	0.90	0.90	0.90	0.90
Digestible P (%)	0.36	0.36	0.36	0.32	0.32	0.32	0.28	0.28	0.28
Na (%)	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Lys (%)	1.11	1.11	1.11	1.02	1.02	1.02	0.98	0.98	0.98
Met + Cys (%)	0.85	0.85	0.85	0.78	0.78	0.78	0.74	0.74	0.74

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg.
[4] Premix Leg 2% (Provimi b.v., Rotterdam, The Netherlands), provides (per kg of diet): vitamin A (retinyl acetate), 12 188 IU; cholecalciferol, 2438 IU; vitamin E (DL-α-tocopheryl acetate), 18.3 IU; pantothenic acid, 42.6 mg; vitamin B_1, 1.2 mg; vitamin B_2, 7.3 mg; vitamin B_3, 9.7 mg; vitamin B_6, 1.2 mg; vitamin B_{12}, 0.024 mg; vitamin K_2, 1.2 mg; folic acid, 0.62 mg; choline chloride, 622.2 mg; calcium, 8784 mg; phosphorus, 3660 mg; sodium, 366 mg; magnesium 36.6 mg; iodine, 0.59 mg; cobalt, 0.59 mg; copper, 2.42 mg; iron, 45.75 mg; manganese, 97.36 mg; zinc, 85.39 mg; selenium, 0.11 mg; methionine, 1647 mg.

Statistical analysis

Data obtained throughout the experiment were analysed by one-way analysis of variance as completely randomized block design with diet as fixed effect and block as random effect using the GLM procedure of SAS[13]. Means were separated by Dunnett's multiple comparison test[14] at $p<0.05$.

RESULTS

Mortality

Mortality rate in the enzymes-supplemented chicken groups (D1 and D2) was statistically similar to that of the control group (C) for the growth phase (d7-d21), the two finisher phases (d22-d28) and (d29-d37), and the entire rearing period (d1-d37; Table 2). This observation is an indication that the dietary incorporation of the enzyme preparations tested here had no deleterious effect on broiler mortality.

Growth performance

At the beginning of the experiment (d7), live body weights were similar for all the groups (Table 3). On d21, the live body weight of broilers fed the reduced energy diet supplemented with Rovabio Excel (D1) was statistically identical ($P = 0.053$) to that of the control birds. Birds on the diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme (D2) had lower (-2.17%) body weight than those

Table 2 - Effect of enzyme dietary supplementation on the broilers mortality.

Diets	Mortality (%)			
	d8-d21	d22-d28	d29-d37	d1-d37
C[1]	1.91[a]	1.29[a]	2.42[a]	8.13[a]
D1[2]	3.39[a]	1.31[a]	1.78[a]	7.94[a]
D2[3]	2.33[a]	0.87[a]	1.34[a]	6.01[a]
SEM[4]	0.93	0.58	0.65	1.70
P-value	0.688	0.626	0.574	0.647

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg.
[4] SEM: standard error of the mean
[a] means within a column with no common superscript differ significantly ($P<0.05$)

Table 3 - Effect of enzyme dietary supplementation on the performance of broilers fed reduced energy diets.

Diets	Live body weight (g)				Daily weight gain (g)			Daily feed intake (g)			FCR		
	d7	d21	d28	d37	d7-d21	d22-d28	d29-d37	d7-d21	d22-d28	d29-d37	d7-d21	d22-d28	d29-d37
C[1]	193.84[a]	1022.7[a]	1657.98[a]	2494.57[a]	59.20[a]	90.75[ab]	92.95[a]	87.34[a]	147.40[a]	184.15[a]	1.47[a]	1.62[b]	1.98[a]
D1[2]	195.10[a]	1022.14[a]	1666.57[a]	2497.32[a]	59.07[a]	92.06[a]	92.3[ab]	86.82[a]	152.63[a]	185.72[a]	1.47[a]	1.66[ab]	2.01[a]
D2[3]	194.51[a]	1000.53[b]	1618.32[b]	2419.62[b]	57.57[a]	88.256[b]	89.03[b]	86.06[a]	148.65[a]	180.15[a]	1.49[a]	1.68[a]	2.03[a]
SEM	9.34	11.46	14.90	2.07	0.62	1.03	1.25	0.97	1.91	3.26	0.03	0.02	0.04
P-value	0.69	0.053	0.048	0.033	0.098	0.047	0.048	0.842	1.169	0.843	0.781	0.028	0.783

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg
[4] SEM: standard error of the mean
[a-b] means within a column with no common superscript differ significantly (P<0.05)

Table 4 - Effect of enzyme dietary supplementation on performance parameters of broilers fed reduced energy diets overall rearing period (1-37 days).

Diets	Initial live body weight (g)	Final live body weight (g)	Daily weight gain (g)	Daily feed intake (g)	Feed conversion ratio	Production index
C[1]	41.78[a]	2494.57[a]	66.29[a]	112.17[a]	1.69[a]	366.5[a]
D1[2]	41.66[a]	2497.3[a]	66.37[a]	113.75[a]	1.71[a]	363.37[a]
D2[3]	41.87[a]	2419.62[b]	64.26[b]	111.19[a]	1.73[a]	355.29[b]
SEM	0.33	2.07	0.4	1.39	0.02	1.08
P-value	1.12	0.033	0.003	0.974	1.41	0.041

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg
[a-b] means within a column with no common superscript differ significantly (P<0.05)

in the control group. A similar trend was observed in live body weights recorded on d 28. At the end of the experiment (d37), the diet D1 resulted in the same final body weight as the control diet, whereas D2 produced broilers with significantly (p<0.05) lower body weight almost 75 g lighter than those fed the control diet (Table 3).

During the grower phase (d7-d21) and the finisher phase 1 (d22-d28), the incorporation of Rovabio Excel (D1) or Natuzyme (D2) into reduced energy diets resulted in weight gain statistically comparable to that obtained with the control diet (Table 3). Throughout the finisher phase 2 (d29-d37), broilers fed D diet grew at the same rate as those given the control diet while broilers fed D2 diet grew at a significantly (p<0.05) slower rate (-4%) than control broilers. A similar trend was observed for body weight gain during the overall growing period (d1-d37) wherein body weight gain of broilers on diet D2 decreased by 3% (Table 4).

Regardless of the growing period, no significant difference was found when comparing feed intake between the control diet and the 2 reduced energy diets supplemented with enzymes (D1 and D2, Tables 3 and 4).

FCR was found to be non-significantly affected among all dietary treatments through the period days 7-21, 29-37 (Table 3) and overall rearing period (1-37 days; Table 4). During days 22-28 of age, the FCR of birds fed the reduced energy diet and supplemented with Rovabio Excel (D1) was equal to that of birds fed the control diet while those of birds fed reduced energy diet but supplemented with Natuzyme (D2) was significantly (p<0.05) higher (+4%; less efficient) than that of control birds (Table 3).

Control chickens had the highest realized value of PI (366,51) followed by those fed the reduced energy diet and supplemented with Rovabio Excel (363,37; Table 4). Both PI values were statistically similar. However, Natuzyme (D2) supplementation decreased significantly (p<0.05) the PI by 3% compared to that achieved with the control diet (Table 4).

Litter quality

No significant difference among dietary treatments was observed, either during the grower phase (d8-d21) or the finisher phases (d22-d28) and (d29-d37; Table 5). Litter quality

Table 5 - Effect of enzyme dietary supplementation on the broilers litter quality.

Diet	Scoring day		
	d21	d28	d37
C[1]	1.81[a]	2.750[a]	3.625[a]
D1[2]	1.88[a]	2.625[a]	3.625[a]
D2[3]	2.00[a]	2.750[a]	3.700[a]
SEM	0.14	0.17	0.16
P-value	0.811	0.79	0.669

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg.
[a] means within a column with no common superscript differ significantly (P<0.05)

Table 6 - Economic analysis of broiler chicken fed reduced energy diets supplemented with enzyme.

Item	Diets		
	C[1]	D1[2]	D2[3]
Feed intake (d1-d7) (kg/bird)	0,209	0,209	0,208
Cost of feed (d1-d7) (TND/kg)	0,7665	0,7541	0,7510
Cost of consummed feed (d1-d7) (TND/bird)	**0,160**	**0,158**	**0,157**
Feed intake (d8-d21) (kg/bird)	1,223	1,215	1,205
Cost of feed (d8-d21) (TND/kg)	0,722	0,7105	0,7049
Cost of consummed feed (d8-d21) (TND/bird)	**0,882**	**0,864**	**0,849**
Feed intake (d22-d28) (kg/bird)	1,032	1,068	1,041
Cost of feed (d22-d28) (TND/kg)	0,7428	0,7412	0,7428
Cost of consummed feed (d22-d28) (TND/bird)	**0,766**	**0,792**	**0,773**
Feed intake (d29-d37) (kg/bird)	1,657	1,671	1,621
Cost of feed (d29-d37) (TND/kg)	0,7249	0,7104	0,7149
Cost of consummed feed (d29-d37) (TND/bird)	**1,202**	**1,187**	**1,159**
Total cost of consummed feed (d1-d37) (TND/bird)	3,011	3,001	2,938
Final live body weight (d37) (kg)	2,495	2,497	2,420
Cost of kg live body weight (TND/kg)	**1,207**	**1,202**	**1,214**

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg
1 USD was equal to about 2.77 Tunisian dinar (TND)

decreased along the days of experiment, independently of litter material, as expected due to the increase in the humidity produced by the birds and their manure.

Economic analysis

The data relative to the economic analysis revealed that the use of reduced energy diet supplemented with Rovabio Excel (D1) decreased the cost per kilogramme of live body weight by 0.44% compared to that of control diet, while that of reduced energy diet and supplemented with Natuzyme (D2) increased this parameter by 0.60% (Table 6).

DISCUSSION

The present paper aims to check whether the individual incorporation of two commercial enzyme products into low energy diet would compensate the reduced energy amount claimed by the producer for broilers fed standard corn-soybean meal based diet. Thus, the two reduced energy diets and supplemented with enzyme products were individually compared to a standard diet (without energy reduction) with respect to mortality, performance parameters and litter quality.

Mortality

Regarding mortality, there was no significant effect of the dietary enzyme inclusion on this parameter. Zanella *et al.*[15] and Hanumantha Rao *et al.*[16] also found that enzymes supplementation of corn-soybean diets did not affect mortality. Similarly, it has been shown that enzyme supplementation of barley-based diet had no significant impact on mortality[17]. However, our finding is not in line with those of Strelec *et al.*[18] and Khan *et al.*[19] who reported that dietary supplementation of broilers with exogenous enzymes decreased mortality rate considerably. In the study conducted by Khan *et al.*[19], broilers were fed a sunflower based diet containing a high fibre level (crude fibre >5%) that caused increasing incidence of pasting vents and wet litter resulting in more coccidiosis cases in control birds. Indeed, without enzymes, indigestible fibre promotes the growth of pathogenic microorganisms but with the enzyme, the fibre is broken down and promotes the growth of beneficial microorganisms.

Growth performance

With respect to growth performance, we investigated whether dietary enzyme supplementation could enable broilers fed reduced energy diets to restore their performance to levels equal to those obtained with a nutritionally adequate diet based on corn and soybean meal (standard diet).

In the current study, no significant differences were found in weight gain, feed intake and FCR of broilers fed diet containing 60 Kcal/kg less ME than the control diet and amended with the Rovabio Excel during all growing phases and over the entire rearing period. This result indicates that birds were able to maintain a weight gain comparable to that of control birds while consuming the same amount of feed but with 60 Kcal/kg less ME than the control diet. Our results are partially consistent with those of Nadeem *et al.*[20] who stated that Rovabio dietary supplementation (0.05 g/kg) of a diet having 50 Kcal/kg less ME than the control diet had no significant effect on weight gain but significantly increased feed intake and decreased FCR during the starter (1-28 days) and overall (1-42 days) growing periods. However, these authors did not observe significant differences in these parameters during the finisher (29-42 days) phase. Recently, in a study with more energy reduction (-100 Kcal/kg diet) than that used in the present study, Govil *et al.*[21] found that when the broilers were fed for 42 days the low energy diet supplemented with NSP degrading enzymes (xylanase at 0.05 g/kg + mannanase at 0.05 g/kg) plus amylase (0.04 g/kg), weight gain and FCR improved significantly, while feed intake was not changed. The study of Khan *et al.*[19] also showed that Rovabio dietary supplementation (0.05 g/kg) significantly improved weight gain and FCR of chicken fed sunflower meal (8%) - corn based diet but did not affect feed intake. The authors also observed a significant improvement in the digestibility of all nutrients in the enzyme-supplemented diet. These findings confirm that the beneficial effects of NSP-degrading enzymes might be somewhat higher with low-digestible feedstuffs like sunflower meal (14-18% crude fiber), than with high-digestible feedstuffs.

The use of an enzyme complex containing carbohydrases and phytase was suggested as a tool to decrease dietary concentration of nutrient, i.e. AME, P, CP/amino acids, and Ca in poultry feeds due to improved nutrient utilisation[9;10; 11;12]. In the present report, Natuzyme supplementation of a diet deficient in energy (-120 Kcal/kg) was successful in fully returning growth performance parameters to those obtained

with a conventional diet without enzymes supplementation during all growing phases excepting for finisher 1 (d22-d28) phase where it resulted in significantly lower body weight gain and thus in significantly higher FCR. Our result regarding non-significant differences in feed intake between Natuzyme supplemented group and the control group is not consistent with those of Attia *et al.*[22] who showed that the supplementation of broilers standard diet with a combination of phytase and multienzyme preparation (containing NSP degrading enzymes and amylase) increased BWG by 4.9%, improved FCR by 6.6%, and decreased feed intake by 2.2%, as compared with those of broilers fed the standard diet without enzyme supplementation. Cowieson and Adeola[23] also reported 14% and 10% improvement in weight gain and FCR, respectively, after supplementing an enzyme cocktail (xylanase, amylase, protease, and phytase) to broilers fed a corn-soybean-based diet that was nutritionally marginal in terms of metabolizable energy (-180 Kcal/kg), Ca, and P. However, in no instance did this multienzyme preparation supplement result in growth performance equal to the nutritionally adequate diet as measured by FCR or BWG. According to authors, this was due more to the scale of the removal of energy and P from the nutritionally marginal diet and not to a lack of response to the supplemented enzymes. Zaghari *et al.*[24] reported that supplementing a corn-soybean meal based diet deficient in metabolisable energy, crude protein, non-phytate phosphorus and amino acids with 0.35 g/kg Natuzyme improved broilers body weight and FCR. However, this supplementation could not restore chick performance to the levels equal to a nutritionally adequate diet. In the same study, the authors estimated nutrient equivalency values of Natuzyme using increased inclusion levels (from 0.1 to 0.4 g/kg) and found that the enzyme product did not liberate nutrient equivalency values recommended by the producer for broilers fed corn-soybean meal based diet. They speculate that these recomended values may be appropriate in diets with high indigestible feedstuffs. In this respect, Makinde *et al.*[25] evaluated the performance of finisher broilers (4 weeks old Anak chickens) fed rice offal (as a replacement of dietary corn) under Natuzyme dietary supplementation. They observed that broilers fed diets containing 20-30% rice offal and supplemented with Natuzyme (0.25 g/kg) had similar final body weight, body weight gain and FCR than those fed corn-soybean meal based diet. Oliaei *et al.*[26] evaluated the effect of supplementing Natuzyme (0.35 g/kg) to broilers diet containing canola meal (6 or 12%) and reported a significant increase in body weight (by 7%) but no effect on feed intake and FCR as compared with the unsupplemented control diets. Recently, the inclusion of different levels of Natuzyme (0, 0.5, 0.75 and 1 g/kg) into a sorghum-based diet did not affect growth performance of Ross 308 broiler chicks, except for FCR which was significantly higher in chickens receiving the highest level of enzyme inclusion during starter phase as compared to control chickens[27]. In the latter study, the enzyme supplemented diets and unsupplemented diet were isocaloric and isonitrogenous.

Litter quality

Improving litter condition reduces ammonia in sheds and reduces the incidence of hock bums and breast blisters, and carcass downgrading in broiler chickens. Feed additives such as exogenous enzymes may have a positive influence in this regard. In the current study, exogenous enzyme dietary supplementation had no effect on litter quality in terms of litter humidity. It has been shown that the dry matter content of the litter of wheat or barley-fed broilers is improved (reduced sticky droppings) by adding NSP degrading enzymes to their diets[28;29]. Yuan *et al.*[30] also reported that NSP hydrolysing enzymes reduced excreta moisture of birds significantly. The results of recent studies showed that diet supplementation with feed enzymes (xylanase, amylase, and protease cocktail) in combination with probiotic bacteria decreased litter moisture and reduced the severity of foot pad dermatitis in broilers[31;32]. However, Cengiz *et al.*[33] reported that the supplementation of a corn-soybean diet with different enzyme preparations (with galactosidase, xylanase, protease, amylase, glucanase, or mannanase activity) had no effect on litter moisture. Similar results were observed when a mixture of NSP-degrading enzymes was added to the diet containing a high level of barley[34]. No improvement was also observed in terms of dry matter content of excreta and litter when carbohydrase complex was added in different inclusion levels in broilers diet[35].

CONCLUSIONS

The findings of this study suggest that supplementation of Rovabio Excel to diet with reduced energy level allowed for the total recovery of broiler growth performance and provided the best economic result. Natuzyme supplementation partially restored performance results. Dietary enzymes did not affect mortality and litter quality. Further studies are needed to consider the impact of summer management and heat stress on energy and nutrient levels with enzyme supplementation since the current study was carried during the spring season. Moreover, this experiment could be repeated in future studies while considering fecal or digesta sampling in order to have much more knowledge about enzyme supplementation effect on chicken gastrointestinal microbiota.

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Challenges of livestock: climate change, animal welfare and agroforestry

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SUMMARY

Livestock is an activity that generates employment and foods for human consumption. The ruminants are fundamental for the conversion of forage resources into foods and traction due to the symbiotic association with the ruminal microbiota. In this context, this manuscript describes the importance of ruminants as generators of resources, but its adverse effects are also described, specifically the emission of greenhouse effect gases (GHG). The growing trend in the demand for beef and sheep meat, as well as bovine milk, suggests analyzing the current strategies used in their feeding and its effect on animal welfare. These strategies have been implemented to increase the productivity per animal unit and, recently, to reduce the intensity of GHG emissions originated by enteric fermentation. However, most of the techniques used to measure the emission of these gases in ruminants are inaccessible in non-development countries, which suggests proposing interdisciplinary strategies to mitigate their emission. Thus, a brief description of agroforestry and its contribution to carbon fixation was also realized. Currents research about non-fixing and nitrogen-fixing were added due to nitrous oxide emissions from forests and Agroforestry Systems. In this way, the livestock agroecosystem and its environmental benefits that favor the mitigation of GHG and animal welfare, are strategies that encourage environmental sustainability and the systems of animal production.

KEY WORDS

Enteric fermentation, one health, ruminants, shrubby, sustainability.

INTRODUCTION

Globally, livestock contributes to human and nutritional needs. It is a source of fertilizer, traction, quality protein, employment and income-generating activity[1], however, livestock also faces big problems arising from its activity, among them, climate change and biodiversity loss[2]. The Food and Agriculture Organization of the United Nations (FAO) expects that there will be a 73% increase in meat and egg consumption and a 58% in dairy consumption by the year 2050 because of both, increase in the population and per capita consumption[3]. In this context, the Organisation for Economic Cooperation and Development and the Food and Agriculture Organization (OECD-FAO)[4] estimates that meat consumers will increase their food intake towards animal protein more expensive, such as beef and sheep meat. The United States, Argentina, India, Mexico, the Russian Federation and Turkey increased its meat production in 2017, which contributes to these growing demands. However, the land use and food production destroy forest and biodiversity[5], which will impact sectors involved in livestock activities to obtain safe and attainable products[4] and will consequently encourage the greenhouses gas (GHG) emissions.

According to the Environmental Protection Agency (EPA)[6], the primary sources of GHG emissions in the U.S. during 2017 were transportation (29%), electricity production (27.5%), industry (22.2%), commercial and residential (12.2%), land use and forestry (11.1%), and agriculture (9.0%). These activities are entwined because the global population grows, urbanizes and consumes more[5]. Nonetheless, agricultural activities, crop and livestock production for food, contribute to emission of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), where each gas's effect on climate change depends on concentration, abundance and Global Warming Potential[6]. In livestock activities, ruminants (cattle, sheep, and goats) are the primary source of CH_4 emission by enteric fermentation and manure fermentation[7]. The global warming potential of CH_4 is 21 to 25 times that of CO_2[8] and its rate of emission is highly dependent on the management strategies implemented on a farm[9]. Regarding enteric fermentation in ruminants, the GHG emission changes according to the production system, management practices, and implemented strategies into their feeding[3;10].

This double challenge, satisfying the food demand without affecting the environment, has led to bear in mind measures

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and strategies for managing land-use and feeding systems together[5]. Some of these actions have shown that reduce or delete products of animal origin for human consumption might reduce the environmental impact, especially, GHG emissions, use of land and water availability[11;12;13]. Other actions suggest intensifying the animal production, which consists in increasing the quantity of product per unit of input in the production system, for example, reducing the necessary area by animal unity and/or their feeding requirements per each unit of produced animal protein. The intensification of animal production systems has been proved as a way to reduce the environmental impact; however, this intensification is associated with reduced animal welfare. Therefore, the relationship between environmental sustainability and animal welfare should not be interpreted as a paradox, but it must be linked to confront the social and environmental problems, the economic and feeding needs in order to achieve food security without affecting the environment, as was suggested by[15]. In this context, the enteric fermentation in ruminants and GHG emissions are described and discussed in the first section, then the methods for measuring the CH_4 emission into these activities are analyzed for their application in developing countries because according their geographic position these countries has available resources for ruminants feeding. Later, the animal welfare and agroforestry are analyzed together as other strategies available for reducing the climate change and the GHG emissions into livestock. The objective was to elucidate the current challenges of livestock and their relationship with animal welfare including agroforestry.

MATERIAL AND METHODS

The methodology of this study consisted of a literature review. The aim of the review was giving a current perspective of literature in the research fields in developing countries. The literature search was performed in 2018 and 2019 with the science search engines Google Scholar and Primo Exlibris into a digital library. The topic were Foods-Livestock and Animal Welfare, the words used by each topic were livestock-methane, methane, greenhouses gas, agroforestry and agroforestry-livestock. The articles included in the review were the direct relationship through the keywords, published in Spanish or English Language and considering the sustainability of livestock systems. The analyses are presented and discussed in this paper.

RESULTS AND DISCUSSION

Livestock activities and GHG emissions

The Framework Convention on Climate Change (UNFCCC), Article 1, defines climate change as "a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods"[15]. The UNFCCC makes a distinction between climate change attributable to human activities, which alters the atmospheric composition, and climatic variability attributable to natural causes.

The anthropogenic emissions of GHG have increased because of population growth and of their economy[6]. This has resulted in a higher concentration of CO_2, CH_4 and N_2O on the atmosphere, such that, the effects of this gasses could be the dominant cause of global warming since half of the 20th century[6]. The GHG emission is carried out from various sectors of the economy (Industry 22%, Agriculture 9%, Transport 28%). Agriculture includes livestock, forestry, and other land uses. Within the livestock, the CH_4 generated by enteric fermentation is the main source of emission and represents around 26% of emitted total[6]. If changes in land use and deforestation (CO_2 emission that represents 10% of the total GHG) were added, these activities must carefully be addressed due to its importance for the production of food, conservation of natural areas and fixation of CO_2.

The contribution to global anthropogenic emissions of GHG are between 7 and 18%, depending on focus and the scope[16,17;18;19;20;21], while the contribution by each continent, of highest to lowest, are Asia, Latin America, Africa, and Europe. The main sources of emission along the agricultural chain are: use and land-use change (forests and natural vegetation replaced by pastures and crops for livestock activities), agricultural activities (fossil fuel used for manufacturing and chemical fertilizers that increase crop yield), animal production (enteric fermentation in ruminants and burning of fossil fuels into farms), and manure management (mainly their storage, application and deposition)[22;23].

The CH_4 and CO_2 are natural by-products produced by enteric fermentation in ruminants from carbohydrates and, to a lesser extent, of the amino acids into the rumen and large intestine of farm animals. The CH_4 is produced in anaerobic conditions by highly specialized methanogenic microorganisms, which are of great interest when some strategies are investigated to reduce their emissions. According to [24] and [25], the highest production of enteric CH_4 in ruminants is carried out into the reticulum-rumen; approximately 13% into the gut and between 2 to 3% in the rectum. The formation of this gas can represent up to 12% of energy consumption in ruminants and is considered an inherent loss in energy metabolism[26].

Thus, to improve the efficiency of energy use in ruminants and reduce GHG emissions, the main strategies to mitigate GHG emissions according to their approach are: 1) to reduce the total emission (inhibit the formation of CH_4 inside of rumen) and 2) to reduce the intensity of emission (decrease the CH_4 emission by each production unity, i.e., without direct methanogenesis)[27]. The first includes chemical inhibitors, electron acceptors (as nitrates), ionophores and lipids within the diet; the second to improve animal health, food digestibility, as well as promoting intensive production systems, reproductive efficiency, and productivity. In summary, strategies that increase the productivity might also reduce carbon footprint generated for livestock. Nevertheless, this will probably be achieved decreasing the animal welfare in intensive systems, although [27] and [28] claims that the supplements used in animal feeding and animal health can also be strategies to reduce the GHG emission and indirectly to improve animal welfare. These strategies encourage environmental sustainability and ethical production by foods.

Uncertainty to measure the CH_4 emission in ruminants

The GHG emission by enteric fermentation in ruminants has been studied by researchers involved in animal nutrition. Their focus has been finding and implementing strategies to

reduce GHG emissions in response to current environmental problems and its effect on climate change. The structure and chemical composition of forages and concentrates used in ruminant feeding are fundamental to understand their fermentation and digestibility, which are related to the GHG emission potential. However, the inventory of GHG emission by enteric fermentation in ruminants may be more precise if the methodologies to measure its emission were able to replicate without problems[8]. According to the Department of Agriculture of the United States, the standard method for measuring the CH_4 emission originated by enteric fermentation in ruminants is by calorimetric respiration chambers[29], although they also describe other techniques that have been used for it, for example, cubes where CH_4 is detected when ruminants introduce their head for eating, internal indicators such as hexafluoride (SF6), micrometeorological methods (integrated horizontal flow, gradient flow), dilution of isotopes, polyethylene tunnels and some new techniques not validated. However, when these methodologies are carried out, the dry matter intake by ruminants is frequently reduced and does not reflect their productivity and feed intake in commercial farms, but the animal welfare decreases and uncertainty increases. Based on the above, some models have also been used to estimate the emission of enteric CH_4 in dairy cattle, but the most of models are based on the intake of metabolizable energy (ME), acid detergent fiber (ADF), and starch content in the diet[29], which suggest more studies about it to strengthen and improve their application.

Procedures to estimate the CH_4 emission potential of forages

The technique of *in vitro* gas production has been used to evaluate the effect of additives, metabolic modifiers, changes in the proportion of ingredients and on the fermentation variables, mainly in ruminant feeding as reported by [30] and Macome[31]. The CH_4 is originated because the fermentation inside rumen and it was not a variable of interest many years ago. Currently, the *in vitro* gas production is used to measure CH_4 generated from the fermentation of substrates and can contribute to estimating the emission potential of CH_4 in forages, feedstuffs, and feeding strategies because of approximately 87% of the methanogenesis takes place within the rumen[32]. According to [33], the chemical composition of the forages and feedstuff, as well as its degradability within the rumen, affect the emission of CH_4 due to the proportion of produced volatile fatty acids. In this context, the *in vitro* gas production is a handy option to characterize the emission potential of CH_4 in ruminants[34], similar to anaerobic digestion, which has been widely used to measure the GHG emission potential of substrates using different inoculum, as anaerobic sludge[35] and animal feces[36]. However, the variability into the methods suggests a careful analysis of the results, as described by [35].

The UNFCCC requires countries to provide estimates of all GHG emissions and their uncertainties using the guidelines of the Intergovernmental Panel on Climate Change[37]. In these guidelines, most of the information generated to estimate the emission of CH_4 by enteric fermentation utilize the Tier 1 and Tier 2 methods. The Tier 2 is a more complex method than Tier 1 and is based on an estimated of the total annual energy intake of a representative animal that then multiplies it by a CH_4 conversion factor (Ym) for specific categories of livestock. For Tier 3, it is necessary to consider the chemical composition of the diet and the concentration of products resulting from its fermentation, so the IPCC suggests to countries with large livestock population to generate emission factors for more precise GHG emissions inventories.

For example, the IPCC provides Ym value of 6.5 ± 1% for dairy cows, cattle fed with agricultural waste and low-quality by-products, grazing cattle, and mature sheep; 4.5 ± 1% for lambs; and 3 ± 1% for finishing cattle consuming less than 900 g concentrate kg/DM. These values decrease when 1 to 4% of fat is added to the diet and increase when the grain concentration decrease[8;38]. However, the use of metabolic modifiers and the addition of some components of essential oils in ruminant feeding can affect these values. For example, the *in vitro* production of CH_4 decreased in response to the addition of garlic oil[39] and mixtures of essential oils[40]. This suggests that the accuracy of the IPCC Tier 2 methodology is low because each food has different GHG emission potential that can be altered by the synergy with other foods and/or the addition of metabolic modifiers or essential oils. It is resulting in great uncertainty to estimate emission inventories of GHG, as argued by [41].

Animal welfare into strategies to reduce the enteric CH_4 emission by ruminants

The animal welfare is a criterion of sustainability, since animals that are raised under production systems that allow them a physical, emotional and behavioral balance, show a better state of health and a utilization of resources more efficient, which improve their productivity and decrease carbon footprint by unit of product[27;42;43].

Animal welfare is a current term of global importance: the global bioethics. This term sets goals very important related with global public health (One Health), self-understanding of culture, and following of social welfare, which together returns towards the bioethics where the preservation of the environment and biodiversity are essential. Recently, a new concept with interconnections between animal welfare, human welfare, and the environment has also been recognized and called "One Welfare"[44;45]. Animal welfare brings significant benefits such as reduced veterinary costs, animal performance, and quality products. Furthermore, it also keeps hygienic standards in the production of foods of animal origin. Animal welfare is strongly related to the health and efficiency of the production of farm animals, and currently, its implementation has increased the commercial value of its products due to the growing number of consumers expect that animal foods are obtained and processed with greater respect towards animals[43]. In this context, some strategies reported in the literature that can be used, depending on the context, to reduce emissions of enteric CH_4 are the following:

The composition of the diet. The type of carbohydrates is important for the production of CH_4 because their fermentation into the rumen can modify pH and consequently alter the microbial population[46]. The starch in the diet promotes the formation of propionate through a change to amylolytic bacteria and a reduction in ruminal pH, which leads to a decrease in methanogenesis[38]. The digestion of the cell wall (mainly hemicellulose) looks with favor on the emission of CH_4 because increase the amount of acetate in relation to propionate. The increase in CH_4 production is due to fer-

mentation towards acetate, which provides a methyl group for methanogenesis[38;46;47]. Therefore, a greater proportion of starch in diets for ruminants tends to decrease the formation of CH_4 and the loss of energy[48]. Nonetheless, higher concentration of soluble carbohydrates in diets for ruminants, especially when it is introduced abruptly and an unsuitable strategy is used, could quickly decrease pH into the rumen and cause ruminal and metabolic acidosis, which eventually is related with hoof problems. Both conditions, acidosis and hoof problems, affect the animal health and have economic consequences to long-term because they are associated with gastrointestinal damage and development of liver abscesses, which decrease the dry matter intake and digestibility[27;49]. In addition, the animals feel pain and consequently, they lose their ability to move and get up freely, which is essential to access feeders and drinking fountain and consequently decrease their production and reproduction. The burden produced by pain avoid the normal expression of animal behavior and affect body condition, fertility, and productivity, which leads to premature aging and is due to a negative effect on the five basic needs of animals[49;50].

Lipids. The emission of enteric methane in ruminant decrease when lipids are used in the feed, but their utilization depends on its cost and their effects on dry matter intake, productivity, and welfare[51]. The medium chain fatty acids reduce methanogenesis by several mechanisms, mainly because they reduce the proportion of energy obtained from fermentable carbohydrates and produce changes in microbial population, particularly when the methanogenic microorganisms are inhibited and unsaturated fatty acids that function as hydrogen acceptors are bio-hydrogenated[52]. The combination of these effects decreases between 3.8 and 5.4% the CH_4 formation in response to the addition of 1 and 6% lipids on dry basis, since it has been reported that higher levels may cause dysbiosis that negatively affects rumen function, dry matter intake and digestibility of non-lipidic energetic feed[53;54]. In conclusion, the addition of large amounts of lipid in ruminant feeding affects their gastrointestinal function, nutritional status, well-being, and productive efficiency, as described by [27] and [28].

Chemical inhibitors. Various chemical compounds inhibit methanogenic microorganisms. Bromochloromethane (BCM), 2-bromo-ethane sulfonate (BES), chloroform and cyclodextrin[27;46], and recently the 3-nitrooxypropanol have been evaluated *in vivo* and are among the most successful[55;56]. Some of these inhibitors reduced CH_4 production up to 50% *in vivo* with a slight reduction in dry matter intake, daily weight gain and feed digestibility in sheep, goats[54;57;58] and beef cattle[56]. However, this potential effect must be contrasted with the risk to human health (through the consumption of products of animal origin), animal health and their effect possible on the environment, in this context [23] mentions that there is a potential risk of toxicity when using halomethanes in ruminants feeding to reduce enteric methane emissions. It's possible effects after a long period of use ranging from liver damage to death. So, considering these harmful side effects of halogenated compounds, it is unlikely that they can be used as routine supplements to mitigate the emission of enteric CH_4 as described by [27]. The addition of 3-nitrooxypropanol decreased from 5 to 24% the CH_4 emission in sheep[59] and from 7 to 59% in cattle[60] with a slight decrease on dry matter intake[55], or when the dry matter intake is restricted to maintenance level[56]. It is relevant to mention that these authors have not reported side effects for health attributable to the administration during 3 to 5 weeks of 3-nitrooxypropanol. In this context, the use of this compound to 14 weeks resulted 30% less CH_4 emission without detecting toxic effects[33], so the use of this inhibitor could be an effective and harmless strategy to mitigate the emission of CH_4, however, more studies focusing on the toxic effects possible are needed as is mentioned by[27].

Ionophores (monensin). It is an antibiotic produced by *Streptomyces cinnamonensis* and routinely used in ruminant feeding. It is related to the reduction up to 30% of enteric CH_4 in response to the addition of 32 to 36 mg/kg body weight in beef cattle and 21 mg/kg body weight in dairy cattle[61;62], while the addition of 10 to 40 mg/kg dry matter has improved food efficiency[61;63]. However, effect decreases between 8 and 10% two to four weeks after it has been used due to the adaptation of the ruminal microflora to this antibiotic[62]. According to [46], monensin reduces methanogenesis through an indirect effect since it affects bacteria producing hydrogen ions and thus leads to a reduction of precursors for methanogenesis. The ionophores improve feed efficiency because decreases dry matter intake without decreases the productivity, in other words, the CH_4 emission per unit of product decreases[64]. The ionophores also are associated with the animal health because its utilization in ruminant feeding has reduced morbidity, mortality and the incidence of subclinical acidosis in feedlot[65]. In contrast to these multiple benefits, ionophores can be toxic in larger doses than recommended. In this context, the global increase in resistance to an antimicrobial represents a major threat to human and animal health because it goes against current human and veterinary medicine and affects food security and the environment. Although the use of ionophores in ruminants feeding could contribute for food security and animal welfare, their improper usage associated with the emergence and spread of antimicrobial-resistant microorganisms represents a great risk[66]. In this way, the European Union prohibits the use of antibiotics as growth promoters given the concern for the bacterial resistance they generate, but outside the European Union, ionophores are still used in ruminants feeding.

Compounds present in plants. Several studies have shown that plants contain a wide variety of secondary compounds with antimicrobial activity that, in certain concentrations, improve ruminal fermentation and decrease the CH_4 emission[10;40;67]. These compounds include mainly tannins, saponins, and some essential oils. However, the reported effects are variable and contradictory due to the concentrations different of ingredients, basal diets and lack of direct comparisons *in vivo*[68;69]. Accordingly, more studies on the utilization of these compounds to identify their toxic effects in short and long term and its potential impact on animal welfare are required, as it is argued by [70].

Agroforestry systems as a strategy to mitigate climate change

Agroforestry was naturally originated in Europe by the interaction of different types of livestock with the surrounding landscape (for example, transhumant system or pig fed with the acorn in Spain). In Latin America, the situation was different, inasmuch as this activity was originated with extensive systems of beef cattle grazed on introduced grassland and

created from the deforestation of large areas of forest in tropical regions. Agroforestry systems are a form of land use that includes the use and exploitation of trees different kinds (timber, fruit, ornamental, planting) combined with crops and sometimes with animals. These systems provide food, fuelwood, bioenergy, medicine, livestock feed, timber, and construction materials, and their contribution climate change mitigation is according to the permanence of carbon sequestration[71]. From the dawn of mankind, when human beings their sedentary life, the combination of trees, crops and livestock into integrated production systems was carried out in a natural way. According to [72], the Agroforestry in Africa was developed as in Latin American such as it is considered an interdisciplinary practice which consist in using the land for productive activities, for example, the association of woody plant species with non-woody plant species, or woody plant species with non-woody plant species and animal species, both of them with variability in the relationship space-time. However, when all are woody species, at least one should also be managed for agricultural production and/or permanent livestock[73]. For example, farmers of Latin American were the first in using tree branches as fences and have given rise to what we now know as living fences, as well, they leave reman of trees for being used as shady by the animals.

The scattered trees in paddocks and some of the components of the live fences are consumed by the cattle, and according to [71] these trees could have low mitigation benefits due to early harvest of its products. *Leucaena leucocephala, Guazuma ulmifolia*, and *Glyricida sepium*, among others, have used the most used and have captured the interest of researchers from different countries, where this phenomenon has been happening. Subsequent to this and in order to utilize the most of species with high protein contents, such as *leucaena*, the concept of protein bank was born, and with it the livestock agroforestry. These plants belong to *Fabaceae* and can fixing atmospheric nitrogen. The nitrogen-fixing and non-fixing trees sequester CO_2[74], which is used into their photosynthesis and indirectly provide foliage to cattle, such that the interaction between agroforestry and livestock can have offsetting effects on the environment, mainly on climate. However, it is very complex because wastes from one system is raw material of the other. Furthermore, current research refers that Nitrogen-fixing trees (leguminous by example) could exacerbate climate change because elevated soil nitrogen driven by the decomposition of nitrogen-rich plant litter can also drive soil emissions of nitrous oxide (N_2O) as have been reported by [74]. The N_2O is a potent greenhouse gas with 300 times more warming potential than CO_2[6] and its atmospheric concentration is dominated by cumulative emissions over the past two centuries such that the benefits of mitigation take much longer time[75]. So, while the ruminant emits CO_2 from enteric fermentation, also intake foliage from trees, encourage nitrogen recycling and probably decrease nitrogen-rich plant litter. An optimal scenario is when the livestock systems incorporates different types of trees (nitrogen fixing and non fixing trees) in different arrangements that include forage banks of different species, as happen currently with *leucaena*. In this way, livestock agroforestry not only is used to feed livestock[76] but also to generate environmental benefits using agroecological principles.

The carbon capture and efficiency of photosynthesis are higher when three or four layers of vegetation are established. The fixing of nitrogen and nutrient recycling have the purpose of increasing biomass production and the organic matter content on the soil[72]. This is feasible because the inputs of silvopastoral systems come mainly from biological processes and not from fossil fuels or synthetic compounds. The intensive silvopastoral systems, such as protein banks or mixed crops, are a good example of intensified agriculture through the natural way to adapt to climate change. So, the increase in the primary productivity of the livestock agroecosystem is due to the existence of more trees, fodder shrubs, weeds and vigorous pastures[76]. In this sense, livestock agroforestry helps capture carbon (or carbon equivalents when the methane is captured or fixed) through vegetative growth (foliage, fruits and roots), and when the ruminants are fed with quality forages the methane emission is decreased, the ruminal efficiency is improved, and the retention of carbon on the soil and losses of nitrogen towards the atmosphere are diminished because of the recycling of excreta is fast and efficient. According to [75], enhanced mitigation of non-CO_2 gases has quantifiable and significant benefits to reduce GHG emissions. In addition to these benefits, the livestock agroforestry provides other environmental services very important as water retention of rain, erosion reduced and recovery of fragmented habit with the consequent return of wildlife ranging from small insects to mammals of medium size. In order to achieve greater productive and environmental benefits, it is recommended the use of various agroforestry species, and with this increase the sustainability of the silvopastoral agroecosystem.

CONCLUSION AND FINAL CONSIDERATIONS

The emission of greenhouse gases by enteric fermentation in ruminants, mainly methane and carbon dioxide, requires improving and implementing emission factors and strategies to mitigate the climatic change. The methodologies used to measure the concentration and the volume of greenhouse gases generated by the enteric fermentation in ruminants are not uniform, so the results can be inconsistent. This has an impact on the strategies that are implemented in the nutrition and feeding of ruminants and may not conform to the guidelines of the IPCC, therefore generating emission factors of greenhouse gases through simpler universal procedures are priorities. The dangers and potential benefits of strategies to mitigate emission of greenhouse gases from livestock activity should be considered during its implementation and must be a priority those that offer both them, improving the environment and animal welfare. In addition, it is essential to consider holistic synchrony between livestock and agroforestry systems, where the conservation of biodiversity, soil and water quality, contribute to the preservation of the habitat for wildlife. In this regard, agroforestry also is a real tool to mitigate climate change. Based on the foregoing, it should be noted that research in animal production has historically been focused on finding technical solutions for problems related to productive efficiency, while animal welfare and environmental sustainability have been studied in isolation. However, the synergies between animal welfare and environmental sustainability occur when improvements in productive efficiency are concomitant, so they must be addressed jointly in the face of the challenge of food security, safety, and preservation of resources.

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Segmental posthetomy in a Murgese stallion: extensive excision with preservation of breeding function

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SUMMARY

The sarcoid is a locally invasive neoplasm and it is described as the most common tumor of equines and having an incidence ranging from 12.9% to 67%. Sarcoids have a significant impact on the health and well-being of Equidae and can represent a serious economic-genetic loss when they affect the reproductive system, particularly in subjects with important blood lines and particular morphological value. We describe a neoformation on the external genitals was observed in a Murgese stallion. The stallion was in excellent general conditions and showed no abnormalities, at general examination, while examining the genital apparatus, a large neoformation on the external genitals was observed. On palpation and ultrasonographic examination, the glans of the genital apparatus were normal. External physical examination showed a hyperkeratotic proliferative-verrucous neoformation, both ulcerated and infected. Clinical appearance was suggestive of proliferative cutaneous lesions as sarcoids and/or summer sores. Surgical treatment was achieved with a segmental posthetomy, performed with the horse anesthetized and in dorsal recumbency, as previously described. The dimensions of the stretched mass were about 22 x 11 centimeters and weighed 600 grams. Histopathological examination allowed a tissue level diagnosis of an equine sarcoid, but the excised mass was also positive to species-specific PCR for Habronema microstoma, a frequent cause of cutaneous and mucocutaneous habronemiasis. After two-year post-surgery the stallion is normally employed in breeding season. The collection of seminal material with an artificial Missouri vagina and during two jumps were carried out one hour apart, whose parameters and their characteristics resulted within physiologic ranges. The fertility data provided were excellent. In the described clinical case, segmental posthetomy, a specific procedure for neoformations of the internal preputial lamina, proved to be valid in the treatment of a large circumferential sarcoid located on the outer lamina of the fold and the preputial ring, with minimal complications.

KEY WORDS

Stallion; preputial lamina; segmental posthetomy; Habronema; mucocutaneous habronemiasis.

INTRODUCTION

The Murgese breed of horse, hailing from the Murge (area in the provinces of Taranto, Bari and Brindisi) is the only Italian breed still bred in purity. The breed currently consists of 4879 individuals[1] distributed throughout Italy. The breed is generally used for country riding, harnessing and dressage, where it is achieving impressive results, giving more importance to animals with high reproductive value.

CLINICAL CASE

Signalment

A Murgese stallion 5 years old registered in the stud book, was referred to the Veterinary Teaching Hospital of the University of Teramo, with an imposing mass against its sheath. The stallion was in excellent general conditions with typical breed characteristics.

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History

The stallion, used for breeding since the age of 30 months, had excellent fertility data, with a foal birth rate of 90%.

Specific examination of the external and internal genital apparatus

Physical examination of the external reproductive tract showed a hyperkeratotic proliferative-verrucous neoformation, ulcerated and infected, surrounding the outer lamina of the fold and the preputial ring but not the inner lamina, the glans or the free part of the rod. There were also other smaller neoformations scattered around the inguinal region (Fig. 1, 2).

Rectal examination, transrectal and transcutaneous ultrasound examination of inguinal lymph nodes were unremarkable.

Removal of the lesions was required due to the impairment of the reproductive activity. The wide extension of the major lesion suggested surgical excision under general anaesthesia.

Treatment

Premedication was achieved with acepromazine (30 mg/kg IM), sedation with medetomidine hydrochloride (7 µg/kg IV) and general anesthesia with ketamine hydrochloride (2.2 mg/kg IV) and diazepam (0.04 mg/kg IV), through a jugular catheter.

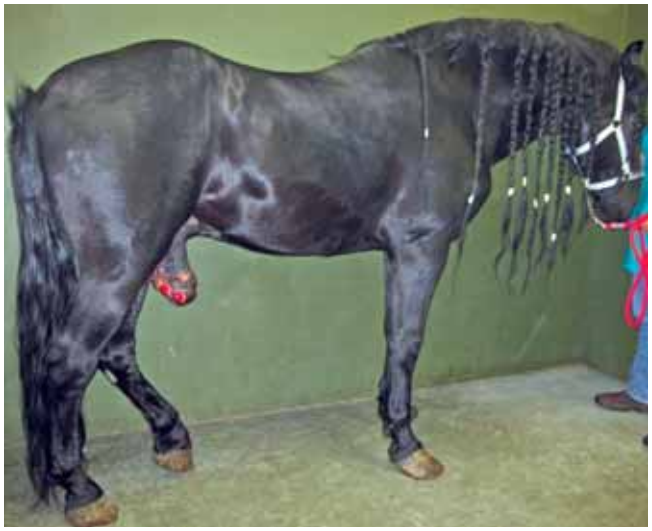

Figure 1 - Subject: Murgese stallion.

Once recumbent, the trachea was intubated and the patient was hoisted onto a surgical table in dorsal recumbency. Maintenance of anaesthesia was obtained with isoflurane and a continuous rate infusion (CRI) of medetomidine (3,5 μg/kg/h IV) and dobutamine (0.5 μg/kg/min IV).

Surgical Technique

After catheterization of the urethra, the penis was extended by traction with a loop of gauze around the collum glandis; the glans and proximal free part of the penis were covered in a sterile bandage, a latex tourniquet was placed at the base of the penile shaft proximal to preputial ring. The penis, prepuce, and the surrounding ventral abdomen were aseptically prepared and the area draped.

Distally and proximally to the lesion, two parallel incisions, through the preputial epithelium were made; the distal circular incision was made at the limit between the inner lamina of the preputial fold and the preputial ring, and the proximal incision at the level of the external preputial orifice. The integument between the incisions was completely removed (Fig. 3), together with the tourniquet, the bleeding vessels cauterized. The subcutis and the skin were sutured with an absorbable monofilament material in a simple interrupted pattern[2].

Aftercare recommendations were systemic antibiotic (sulphamidic 30 mg/kg PO), and antinflammatory therapy (suxibuzone 3,3 mg/kg PO), stall rest for 5 days, isolation from mares for 2-4 weeks and wearing a stallion ring for at least 2 weeks. Regular exercise (10-15 min of daily hand walk) was also suggested.

The stretched mass measured 22 x 11 cm and weighed 600 gr.

Figure 2 - External physical examination.

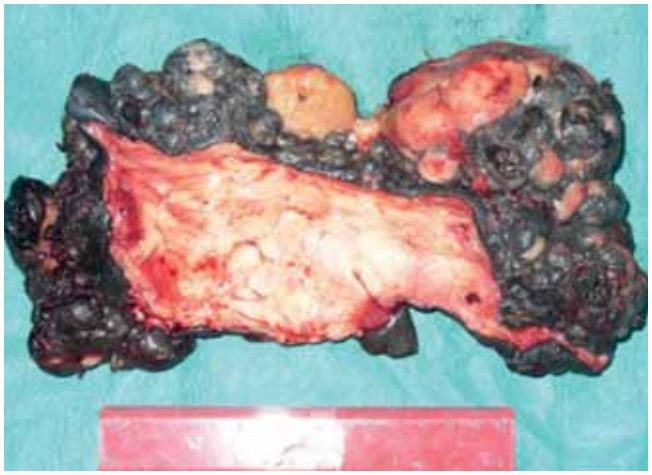

Figure 3 - Stretched mass after excision.

Complications included partial suture dehiscence and a mild colic syndrome. The minor neoformations were excised at the same time along with the application of a cytotoxic ointment. Histopathological examination allowed a diagnosis of an equine sarcoid, and a positivity to PCR for Habronema microstome.

No recurrences occurred in 2 years follow-up. The stallion was regularly employed for reproduction, and all the phases of the physiological mating were carried out regularly.

The collection of seminal material allowed the macro- and microscopic evaluation of the seminal material and the characteristics of the urethral pulse at the beginning of two breeding seasons following surgery (years 2006 - 2007); for semen collection, two jumps were carried out one hour apart. Seminal characteristics in 2006 and 2007 are shown in Table 1[3].

Table 1 - Semen parameters and their characteristics, respectively for the years 2006 and 2007.

Year 2006			Year 2007		
Subject	**1° collection**	**2° collection**	**Subject**	**1° collection**	**2° collection**
Reaction time (min.)	3 minutes	5 minutes	Reaction time (min.)	2	4
Volume before filtration (ml)	90	70	Volume before filtration (ml)	70	75
Volume gel-free volume (ml)	75	60	Volume gel-free volume (ml)	60	64
Color	milky	milky	Color	milky	
Smell	heated metal	heated metal	Smell	heated metal	heated metal
pH	7,3	7,3	pH	7,2	7,1
Motility (%)	80	85	Motility (%)	80	85
Vitality (%)	90	90	Vitality (%)	95	90
Concentration (X 10^6/ml)	320	210	Concentration (X 10^6/ml)	330	325
Total number of sperm for ejaculate (X 10^9)	24	12,600	Total number of sperm for ejaculate (X 10^9)	19,800	20,800
Room temperature motility (h)	2	2,30	Room temperature motility (h)	3,30	3,00
Primary morphological abnormalities (%)	7	6	Primary morphological abnormalities (%)	8	7
Secondary morphological abnormalities (%)	11	10	Secondary morphological abnormalities (%)	25	6

DISCUSSION AND CONCLUSIONS

Segmental posthetomy, a specific procedure for neoformations of the internal preputial lamina, is indicated for the removal of preputial neoplasms, granulomas or extensive scars. This method allows the conservation of the rod, if the lesion does not affect the underlying albuginea tunic. The procedure is described in geldings; whereas there are few reports in active stallions, above all for the major concern about the secondary impotence[4].

The sarcoid is a locally invasive neoplasm, with an incidence ranging from 12.9% - 67%[5]. Sarcoids have a significant impact on the health and well-being of Equidae and can represent a serious economic-genetic loss when they affect the reproductive system, if affecting subjects with important geneaology and morphological value. The absence of significative findings at rectal and ultrasound examination of inguinal lymph nodes reflects the locally invasiveness of the disease[6]. In addition, inguinal lymph nodes are difficult to explore in the horse and often not macroscopically reactive until the later stages of neoplastic conditions[7-10].

In the described clinical case, segmental posthetomy proved to be a valid treatment of a large circumferential sarcoid located on the outer lamina of the fold and the preputial ring, with minimal complications.

For equine sarcoids there are no treatments that are described as 100% effective. Combining different treatment modalities the results can be improved and recurrences can be reduced[6].

From the literature examined, there are no reports on the successful treatment of such extended sarcoids of the external genitalia in breeding subjects[11].

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