### **UNIVERSIDADE FEDERAL DE MINAS GERAIS**

Escola de Medicina Veterinária

Programa de Pós-Graduação em Zootecnia

Alan Figueiredo de Oliveira

# DESEMPENHO DE BOVINOS E EMISSÃO DE METANO EM SISTEMAS SILVIPASTORIS EM REGIÕES DE CLIMA TROPICAL

Belo Horizonte

Alan Figueiredo de Oliveira

### DESEMPENHO DE BOVINOS E EMISSÃO DE METANO EM SISTEMAS SILVIPASTORIS EM REGIÕES DE CLIMA TROPICAL

Tese apresentada ao Programa de Pós-Graduação em Zootecnia da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do título de Doutor em Zootecnia.

Orientadora: Profa. Dra. Ângela Maria Quintão Lana Coorientador: Prof. Dr. Lúcio Carlos Gonçalves Coorientador: Dr. Roberto Guimarães Júnior

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Para concluir o Doutorado, o(a) candidato(a) deverá entregar 03 volumes encadernados da versão final da tese acatando, se houver, as modificações sugeridas pela banca, e a comprovação de submissão de pelo menos um artigo científico em periódico recomendado pelo Colegiado dos Cursos. Para tanto terá o prazo máximo de 60 dias a contar da data defesa.

O resultado final, foi comunicado publicamente ao (a) candidato (a) pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora e encaminhada juntamente com um exemplar da tese apresentada para defesa.

Belo Horizonte, 20 de julho de 2023.

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#### **RESUMO**

Os sistemas silvipastoris (SSPs) podem conciliar altos níveis de produção e preservação ambiental, o que torna fundamental definir recomendações práticas para sua implementação e o impacto ambiental desses sistemas. Objetivou-se (i) comparar a emissão de metano entérico e desempenho de vacas leiteiras pastejando sistemas integrados no Brasil, (ii) avaliar as características do pasto e o desempenho de bovinos em SSPs com Eucalyptus e Urochloa por meio de revisão sistemática e metanálise e (iii) avaliar o efeito do sombreamento sobre as características de gramíneas tropicais e o desempenho de bovinos em SSPs por meio de revisão sistemática e metanálise. No primeiro experimento dezoito vacas holandês-zebu foram distribuídas aleatoriamente em sistema de integração lavoura-pecuária ou em sistema de integração lavoura-pecuária-floresta para determinação da produção de leite, eficiência alimentar, consumo e emissão de metano. A produção de leite e a eficiência alimentar foram semelhantes entre os sistemas. A perda de energia e a emissão de metano foram iguais entre os sistemas, o que mostra não haver diferenças significativas entre os sistemas. No segundo experimento pesquisas sistemáticas em bases de dados encontraram 2.639 artigos, dos quais 29 (120 comparações) que avaliaram SSPs com Eucalyptus spp. e Urochloa spp. foram selecionados. A massa de forragem foi maior nos SSPs com até 99 árvores/ha e menor com as demais densidades em relação ao pasto em monocultivo. A massa de forragem foi menor nos SSPs com todos os espaçamentos entre linhas de árvores e orientações de plantio em relação ao pasto em monocultura, sendo observado menor massa de forragem com menor espaçamento e com orientação de plantio norte-sul. O ganho de peso total por área (GPT) foi menor nos SSPs com menos de 28m entre renques de árvores ou com mais de 199 árvores/ha, mas foi maior em SSPs com mais de 28m entre renques ou com até 99 árvores/ha em comparação com o pasto em monocultura. O GPT foi menor nos SSPs com orientação de plantio norte-sul em comparação com o pasto em monocultura, mas foi igual nos com orientação leste-oeste. No

terceiro experimento foram selecionados 66 artigos com todos os tipos de árvores e gramíneas tropicais. Houve uma pequena redução na massa de forragem em SSPs com árvores leguminosas, mas o ganho de peso por área foi semelhante ao pasto em monocultivo. Os animais também apresentaram maior consumo de matéria seca, consumo de proteína bruta e produção de leite nesses SSPs com árvores leguminosas. As gramíneas tropicais nos SSPs com palmeiras apresentaram maior proteína bruta, menor massa de forragem e não houve redução no GPT em relação ao pasto em monocultura, o que indica a possibilidade de produção animal juntamente com palmeiras. Os SSPs com outros tipos de árvore tiveram maior GPT em relação ao pasto em monocultivo. Esse resultado indica que o uso de SSPs com árvores nativas pode integrar a produção animal com a preservação ambiental. SSPs com ganho de peso por área maior ou semelhante em comparação com o pasto em monocultura podem aumentar a produção total do sistema e a lucratividade.

Palavras-chave: agrofloresta, eficiência produtiva, *Eucalyptus*, sistemas integrados, *Leucaena leococephala*, *Megathyrsus*, metano, *Urochloa*.

### ABSTRACT

Silvopastoral systems (SSPs) can reconcile high levels of production and environmental preservation, which makes it essential to define practical recommendations for their implementation and the environmental impact of these systems. This study aimed (i) to compare the enteric methane emission and the performance of dairy cows grazing integrated systems in Brazil, (ii) to evaluate the pasture characteristics and the performance of cattle in SSPs with Eucalyptus and Urochloa through systematic review and meta-analysis and (iii) to evaluate the effect of shading on tropical grass traits and cattle performance in SSPs through systematic review and meta-analysis. In the first experiment, eighteen Holstein-Zebu cows were randomly assigned to a crop-livestock integration system (CLI) or a crop-livestock-forest integration system (CLI) to determine milk production, feed efficiency, consumption and methane emission. Milk production and feed efficiency were similar between systems. Energy loss and methane emission were the same between the systems, which shows that there are no significant differences between the systems. In the second experiment, systematic searches in databases found 2,639 articles, of which 29 (120 comparisons) evaluated SSPs with *Eucalyptus* spp. and Urochloa spp. were selected. The forage mass was higher in the SSPs with up to 99 trees/ha and lower with the other densities in relation to the pasture in monoculture. Forage mass was lower in SSPs with all spacing between tree lines and planting orientations in relation to pasture in monoculture, with lower forage mass being observed with smaller spacing and with northsouth planting orientation. Total weight gain per area (GPT) was lowest in SSPs with less than 28m between rows of trees or with more than 199 trees/ha, but was higher in SSPs with more than 28m between rows or with up to 99 trees/ha in comparison with pasture in monoculture. GPT was lower in SSPs with north-south planting orientation compared to monoculture pasture, but was equal in east-west orientation. In the third experiment, 66 articles were selected with all types of tropical trees and grasses. There was a small reduction in forage mass in SSPs with

leguminous trees, but weight gain per area was similar to pasture in monoculture. The animals also had higher dry matter intake, crude protein intake and milk production in these SSPs with leguminous trees. Tropical grasses in SSPs with palm trees showed higher crude protein, lower forage mass and there was no reduction in GPT compared to pasture in monoculture, which indicates the possibility of animal production together with palm trees. SSPs with other tree types had higher GPT compared to pasture in monoculture. This result indicates that the use of SSPs with native trees can integrate animal production with environmental preservation. SSPs with greater or similar weight gain per area compared to monoculture pasture can increase total system production and profitability.

Keywords: agroforestry, productive efficiency, *Eucalyptus*, integrated systems, *Leucaena leococephala, Megathyrsus*, methane, *Urochloa*.

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# 1. Capítulo 1: REVISÃO DE LITERATURA - EMISSÃO DE METANO E DESEMPENHO DE BOVINOS EM SISTEMAS SILVIPASTORIS EM REGIÕES DE CLIMA TROPICAL

### 1.1. INTRODUÇÃO

Os sistemas silvipastoris (SSPs) são compostos por diferentes animais, pastagens e árvores na mesma área, sendo os formados por bovinos, *Eucalyptus sp.* e *Urochloa sp.* os mais utilizados no Brasil. Essa integração entre diferentes espécies gera interações sinérgicas positivas dentro do sistema, o que pode melhorar o desempenho produtivo e os indicadores ambientais. Entre esses efeitos sinérgicos positivos podem ser citados a redução do índice de temperatura e umidade (ITU) e da radiação direta que é gerado pelas árvores, o que indica melhoria do ambiente e do conforto para os animais, a melhoria do valor nutricional das pastagens que crescem em sombreamento e a melhoria da matéria orgânica do solo a partir da decomposição dos desejos dos animais. Além desses efeitos sinérgicos, o sombreamento das pastagens e a competição por água e nutrientes também alteram as características do pasto e o desempenho animal. As pastagens em SSPs normalmente contêm maior teor de proteína bruta (PB), são mais altas e menos densas em comparação com o pasto em monocultura, além de produzirem menos em sistemas com alta densidade arbórea (Santos et al., 2016; Santos et al., 2018; Santos et al., 2023). Essas alterações nas pastagens juntamente com alterações no ambiente do sistema produtivo podem alterar o desempenho animal e financeiro desses SSPs.

O valor nutricional das gramíneas tropicais em SSPs varia devido ao crescimento das plantas em ambiente sombreado. As plantas em sombreamento normalmente passam por menor estresse metabólico e permanecem em estado fisiológico mais jovem, o que aumenta de forma consistente os teores de PB (Santos et al., 2023). Por outro lado, alterações morfológicas como maior altura e menor proporção de folhas fazem com que os teores de fibras e de digestibilidade

não apresentem um padrão de variação bem definido. Essas alterações morfológicas além de alterarem o valor nutricional do pasto, também alteram a estrutura e a dinâmica de pastejo dos animais (Geremia et al., 2018; Gomes et al., 2019). Além de alterações importantes no valor nutricional e na estrutura do pasto, o sombreamento nos SSPs também melhora o ambiente para os animais, com reduções do índice de temperatura e umidade e da radiação solar direta incidente sobre os animais (Giro et al., 2019; Gomes et al., 2020). Essas melhores condições podem melhorar o desempenho dos bovinos nesses SSPs, além de aumentar a eficiência e reduzir as emissões de gases de efeito estufa, como o metano.

O desempenho de bovinos em SSPs é influenciado por diversos fatores como a qualidade e estrutura do pasto, o conforto térmico, mas principalmente pela disponibilidade do pasto. Em sistemas com alta densidade arbórea o pasto normalmente apresenta pior estrutura e menor oferta de forragem, o que pode reduzir o ganho de peso por área em bovinos de corte (Santos et al., 2018). Entretanto, estudos recentes com menor densidade arbórea mostraram que o desempenho pode ser maior devido a maior oferta e qualidade do pasto e a melhoria do conforto térmico (Magalhães et al., 2018; Carvalho et al., 2019; Domiciano et al., 2020). Já em rebanhos leiteiros os dados de desempenho são mais escassos. Martins et al. (2020) observaram produções semelhantes de leite em vacas mantidas em SSPs ou em pasto em monocultura. Entretanto, a redução significativa da capacidade de suporte dos pastos em SSPs com alta densidade arbórea também provavelmente reduz a produção de leite por área.

As alterações no microclima local e na ciclagem de nutrientes dentro dos SSPs também podem alterar os indicadores ambientais. Os estoques de carbono no solo por exemplo normalmente são maiores nos SSPs em comparação com o monocultivo de pasto (Moreira et al., 2022), o que pode mitigar as emissões de gases do efeito estufa (GEE) do sistema. Figueiredo et al. (2016) mostraram que esse aumento nos estoques de carbono reduziu a pegada de carbono de 18,5 kg CO<sub>2</sub> eq./kg de peso vivo (PV) em pasto degradado para -28,1 kg CO<sub>2</sub> eq./kg PV em SSP. Além disso, a melhoria do valor nutricional pode melhorar a fermentação ruminal dos animais criados em pasto e reduzir a emissão de metano entérico. Assim, o objetivo desse capítulo foi discutir a literatura sobre os principais indicadores produtivos e ambientais de SSPs em regiões de clima tropical.

## 1.2. CARACTERÍSTICAS AGRONÔMICAS DE GRAMÍNEAS TROPICAIS EM SISTEMAS SILVIPASTORIS

A presença das árvores nos SSPs reduz a radiação fotossinteticamente ativa (RFA) que atinge o pasto, o que altera o microclima local e a produtividade e morfologia das plantas forrageiras. Pezzopane et al. (2015), por exemplo, encontraram maior RFA na pastagem em monocultura (7,6 MJ/m<sup>2</sup>/dia) em relação a dois (4,6 MJ/m<sup>2</sup>/dia) e a 8,5 metros a partir do renque das árvores (7,0 MJ/m<sup>2</sup>/dia) em um SSP formado pela *U. decumbens* cv. Basilisk e árvores nativas no Brasil. A densidade e as características das espécies arbóreas são os principais fatores que podem reduzir a produção das forrageiras (Pezzopane et al., 2020). Os SSPs mais adensados apresentam reduções acentuadas das produtividades das pastagens (Gomes et al., 2019). Em sistemas que utilizam espaçamentos maiores, a redução da produtividade geralmente não é significativa (Nascimento et al., 2019). Assim, o planejamento desse sistema deve considerar as estratégias comerciais do empreendimento rural para definir os arranjos técnicos do sistema a fim de beneficiar a pecuária com maiores espaçamentos ou a silvicultura com menores espaçamentos.

A conciliação entre o máximo benefício do sombreamento em relação ao aumento do teor proteico do pasto e a redução da taxa de acúmulo de forragem é extremamente difícil em SSPs. O sombreamento superior a 30% a até 6 m de distância do renque das árvores propiciou maior teor proteico na forragem, mas foi prejudicial ao perfilhamento e ao acúmulo de matéria seca de forragem (Paciullo et al., 2011). Em pastagens de *Urochloa* sp. com sombreamento moderado (redução de 25 a 35% da RFA), têm-se obtido produções de forragem semelhantes

ou maiores do que em pastagem em monocultura. Nessas condições, as plantas apresentam ajustes morfofisiológicos, como aumentos da relação parte aérea/raiz e da área foliar específica que permitem a manutenção da produtividade.

Oliveira et al. (2014) encontraram produções de matéria seca (MS) na *U. brizantha* cv. Piatã de 7.274 kg MS/ha na pastagem em monocultura, de 4.781 kg MS/ha no SSP formado com eucalipto com 22 m entre renques e de 3.441 kg MS/ha no SSP com 14 m entre renques, o que representa reduções de 34,3 e 52,7%. Já Santos et al. (2016) observaram redução de 42,8 kg MS/ha de forragem na *U. brizantha* cv. Piatã a cada 1% de redução de RFA. O acúmulo de massa seca de forragem no período chuvoso foi maior na pastagem em monocultura em relação ao SSP formado com 12 e com 22 metros entre renques, o que representa reduções de 67,5% e 50,5%, respectivamente. A proximidade dos renques de eucalipto, a competição por água, a redução da RFA e o plantio no sentido norte-sul foram citados como motivos para essa redução.

Entretanto, estudos mais recentes com menores densidades arbóreas e maiores espaçamentos entre renques mostraram produções semelhantes ou maiores nos SSPs em relação ao pasto em monocultura (Domiciano et al., 2020). Magalhães et al. (2018) observaram acúmulo de forragem 60% maior na *U. brizantha* cv. Marandu em um SSP com 30 m entre renques em comparação com o pasto em monocultura (13,410 vs. 21,430 kg MS/ha/ano). Carvalho et al. (2019) também observaram aumento no acúmulo de forragem em SSP com 37 m entre renques de eucalipto em comparação ao pleno sol (24,050 vs. 19,500 kg MS/ha/ano). Esses dados mostram a necessidade de aumentar o espaçamento entre renques em sistemas onde se objetiva maximizar o desempenho animal.

Paciullo et al. (2016) observaram redução do perfilhamento com o aumento do sombreamento no *Megathyrsus maximum* cv. Massai e cv. Tanzânia. Essa redução de perfilhamento em SSPs acontece devido a priorização da planta em alocar energia para o crescimento vertical para captar mais luz em detrimento da emissão de novos perfilhos. As

forrageiras cultivadas sob sombreamento tendem a aumentar a proporção e o tamanho dos colmos e suas alturas. Esse comportamento foi observado por Geremia et al. (2018) na *U. brizantha* cv. Piatã em pastagem em monocultura que foi mais baixa e apresentou maior relação folha/colmo em relação ao SSP com média e alta densidade de árvores de eucalipto. Essa variação pode ser atribuída ao estiolamento da planta em condições de sombreamento para buscar mais luz. Santos et al. (2018) também atribuíram a menor participação da folha no SSP ao estiolamento da planta. Além disso, as alturas pós-pastejo foram maiores nos SSPs, que, junto ao menor volume de folhas, geraram pior dinâmica de pastejo para os animais e dificultaram o manejo do pasto.

Santos et al. (2016) observaram maior índice de área foliar na *U. brizantha* cv. Piatã em pastagem em monocultura (2,5) em relação ao sistema com 12 metros entre renques (1,6), provavelmente devido à menores densidade de perfilhos e produtividade por área. Entretanto, a área foliar específica foi maior no SSP com 12 metros (184,6 cm<sup>2</sup>/g) em relação ao SSP com 22 metros (162,3 cm<sup>2</sup>/g) e a pastagem em monocultura (145,3 cm<sup>2</sup>/g). Em condições de sombreamento, as folhas apresentam menos tecido de suporte e menor número de células mesófilas por unidade de área, o que resulta em folhas mais finas e com maior área foliar específica. Essas alterações morfológicas objetivam compensar a deficiência de luz e manter a capacidade fotossintética da planta.

Santos et al. (2018) observaram densidade de forragem de 96 kg/ha a cada cm de altura do pasto em monocultura, de 58 kg/ha a cada cm de altura do pasto no SSP com 22 metros entre renques e de 38 kg/ha a cada cm de altura do pasto no SSP com 12 metros entre renques. A densidade na pastagem em monocultura foi 65,5 e 152,6% maior em comparação ao SSP com 22 e 12 metros entre renques. A menor densidade de forragem em sistemas sombreados ocorre em razão da maior altura, do menor número de perfilhos e da menor produção por área. Essas características podem influenciar o comportamento de pastejo e o desempenho dos animais, uma vez que estes passam mais tempo caminhando à procura de forragem no pasto.

### 1.3. VALOR NUTRICIONAL DE GRAMÍNEAS TROPICAIS EM SISTEMAS SILVIPASTORIS

A avaliação do valor nutritivo é fundamental para o estabelecimento de um adequado programa nutricional nos sistemas de produção que utilizam pastagens. A melhoria do valor nutritivo das pastagens vem sendo considerada como uma das grandes vantagens dos SSPs. A qualidade nutritiva das forrageiras pode ser influenciada pela maior ciclagem de nutrientes no solo, pela alteração do microclima local e pelas alterações na morfofisiologia das plantas. Além do valor nutritivo, é preciso considerar que a redução da produtividade das pastagens em locais sombreados pode diminuir a produção de nutrientes, a capacidade de suporte e a produção animal nessas áreas (Lima et al., 2018; Lima et al., 2019).

O teor de PB é o principal componente nutricional da forragem que sofre alterações em condições sombreadas, com aumentos significativos devido a maior ciclagem de nutrientes no solo, do padrão de desenvolvimento das plantas e da maior concentração de nitrogênio na planta devido à menor produção de tecidos (Pezzopane et al., 2020). Em pastagens com maiores teores de PB, a menor necessidade de suplementação proteica pode reduzir os custos de produção dos sistemas. Assim, forrageiras com maiores teores de PB são desejáveis como opção para reduzir custos.

Lima et al. (2018) avaliaram por dois anos a *U. decumbens* em SSP com 30 m entre renques de árvores de eucalipto e observaram teor de PB 29% maior em SSPs (109 e 128 g/kg) em comparação com a monocultura do pasto (87 e 96 g/kg). Entretanto, como a produção do pasto foi reduzida, foi observado maior produção de PB por área na pastagem em monocultura. Santos et al. (2018) encontraram menores teores de PB (8,3%) na pastagem em monocultura em relação ao SSP com *U. brizantha* cv. Piatã e renques de eucalipto em espaçamento de 22 m

(10,7%) e no SSP com 12 m entre renques (11,7%), aumentos de 28,9 e 41,0%. A produção de PB foi de 1.137 kg/ha/ano na pastagem em monocultura, de 804 kg/ha/ano no sistema com 22m entre renques e de 582 kg/ha/ano no sistema com 12m entre renques. Essa redução da produção de PB demonstra que, mesmo com maiores teores nos sistemas sombreados, a capacidade produtiva do pasto é maior na pastagem em monocultura devido à maior produtividade de nutrientes por área.

Paciullo et al. (2016) encontraram maiores teores de PB no *M. maximum* cv. Massai e cv. Tanzânia submetidos a 58% de sombra em comparação a pastagem em monocultura. Além disso, o aumento da dose de nitrogênio de 50 para 150 kg/ha também elevou o teor de PB da planta. Os teores de nitrogênio insolúvel em detergente neutro foram maiores no *M. maximum* cv. Massai com 58% de sombra. Dessa forma, os maiores teores de PB em plantas sombreadas podem não resultar em maiores teores de PB utilizadas pelo animal.

O maior teor de PB nas forragens sob sombra pode ser explicado pela teoria da diluição de nitrogênio. Conforme essa teoria, as forrageiras em um nível similar de produção extraem uma porcentagem parecida de nitrogênio do solo. Assim, com a maior produção de biomassa das plantas na pastagem em monocultura, há uma diluição no nitrogênio absorvido e translocado para as partes aéreas em comparação com as plantas cultivadas em áreas sombreadas. As plantas sob sombra não metabolizam e convertem todo o nitrogênio absorvido em acúmulo de MS e, dessa forma, o nitrogênio fica concentrado e aumenta o teor de PB da planta. Os acréscimos nos teores de PB nas plantas em sombreamento natural também podem estar ligados ao aumento da degradação da matéria orgânica e da reciclagem de nitrogênio no solo (Moreira et al., 2022). Além disso, o atraso no desenvolvimento ontogênico das plantas cultivadas à sombra mantém as plantas mais jovens fisiologicamente, o que possibilita alta taxa metabólica da célula e menor acúmulo de fibra.

Os teores de fibras e a digestibilidade das forragens não apresentam um padrão de variação definido. Em algumas situações, o estiolamento da planta em sombreamento pode aumentar as frações fibrosas e reduzir a digestibilidade. Já em outras, os aumentos de PB e alterações na parede celular podem reduzir os teores de fibras e aumentar a digestibilidade. Paciullo et al. (2016) avaliaram o *M. Maximum* cv. Massai e cv. Tanzânia em diferentes intensidades de sombreamento e encontraram menores teores de fibra em detergente neutro (FDN) com os aumentos de sombreamento. Lima et al. (2018) também observaram menor teor de FDN na *U. decumbens* no SSP (65,8%) em comparação a pastagem em monocultura (67,7%). Já Paciullo et al. (2014) não constataram diferença nos teores de FDN na *U. decumbens* consorciada com estilosantes em pastagem em monocultura e no SSP.

Santos et al. (2018) encontraram teores similares de FDN, fibra em detergente ácido (FDA), celulose, hemiceluloses e lignina na *U. brizantha* cv. Piatã em pastagem em monocultura e no SSP com 12 e com 22 m entre renques. Entretanto, essas frações foram maiores no período chuvoso, com valores de 66,8, 32,7, 29,5, 34,1 e 2,8%, respectivamente. Segundo os autores, os maiores teores de fibras no verão podem estar associados ao maior acúmulo de forragem causado pelas condições climáticas, que favorecem o alongamento das folhas e o aumento do conteúdo dos componentes estruturais para manter a arquitetura foliar. Geremia et al. (2018) também não encontraram diferença nos teores de FDN e de FDA na *U. brizantha* cv. Piatã em pastagem em monocultura e no SSP com média e alta densidade de árvores.

A falta de um padrão de variação está ligada a algumas alterações na planta, que podem aumentar, reduzir ou manter os teores de fibras em determinadas condições. As plantas em sombreamento tendem a estiolar com o avanço da maturidade como estratégia para aumentar a altura e buscar luminosidade, o que pode resultar em alongamento do colmo e aumento nos teores de fibra da forrageira (Santos et al., 2016). Entretanto, outros fatores, como o aumento do teor de PB, a alteração no desenvolvimento ontogenético e a redução da espessura da parede celular, podem reduzir os teores de fibras das forragens que, geralmente, não apresentam alterações consistentes desses componentes (Santos et al., 2023). Gómez et al. (2012) citaram que as folhas das forragens sob baixa incidência de luz apresentam menos tecido de sustentação e menor número de células mesófilas por unidade de área, o que produz folhas mais finas e gera menores teores de fibras.

De forma semelhante ao teor de fibra, a digestibilidade das pastagens também não apresenta um padrão de variação bem definido. Paciullo et al. (2014) não verificaram diferença da digestibilidade *in vitro* da matéria seca (DIVMS) na *U. decumbens* consorciada com *Stylosanthes guianensis* em pastagem em monocultura e no SSP com 30 m entre renques e consorciada com *Stylosanthes guianensis*, *Pueraria phaseoloides* e *Calopogonium mucunoides*. Geremia et al. (2018) também não observaram diferença na DIVMS da *U. brizantha* cv. Piatã em pastagem em monocultura, no SSP com média e alta densidade de árvores. Já Santos et al. (2018) observaram maior DIVMS na *U. brizantha* cv. Piatã na pastagem em monocultura em comparação ao SSP espaçado de 12 m entre renques (65,8 vs. 62,3%). Os autores atribuíram a maior digestibilidade da forrageira na pastagem em monocultura à maior relação folha/colmo e à fibra de melhor qualidade.

Na mesma área experimental, Santos et al. (2016) constataram produção de matéria seca digestível na pastagem em monocultura de 9.019 kg/ha/ano, no SSP com 22m entre renques de 4.858 kg/ha/ano, e no SSP com 12m entre renques de 3.103 kg/ha/ano. Essa redução acentuada na produção de matéria seca digestível nos SSPs com árvores mais adensadas também foi responsável pelo menor ganho de peso animal por área devido à menor oferta de forragem.

### **1.4. DESEMPENHO DE BOVINOS DE CORTE EM SISTEMAS SILVIPASTORIS**

Os SSPs propiciam o aumento do conforto e do bem-estar animal, o que pode melhorar a eficiência produtiva. A redução da produção dos pastos em sistemas com alta densidade de árvores afeta negativamente a capacidade de suporte e o ganho de peso dos animais. Porém, em sistemas com menores densidades arbóreas o desempenho pode ser aumentado nesses sistemas. Além disso, a alteração das características agronômicas das pastagens pode piorar a dinâmica de pastejo dos animais, que dispendem mais tempo se deslocando em busca de forragem.

Oliveira et al. (2014) avaliaram o desempenho animal em pastagem de *U. brizantha* cv. Piatã em pleno sol e em SSPs com 14 ou 22 m entre renques, manejada com duas alturas de pastejo. Não houve diferença no ganho de peso médio diário dos animais entre os tratamentos. Entretanto, a capacidade de suporte foi menor durante todo o ano no SSP com 14 m em relação aos demais tratamentos e menor no outono no SSP com 22 m em comparação ao pleno sol. O ganho de peso vivo por hectare em cada estação foi menor no SSP com 14 m e semelhante entre o pleno sol e o SSP com 22 m. Porém, o ganho de peso total durante o ano foi de 537 kg/ha (17,9 @ de PV) no pleno sol, de 459 kg/ha (15,3 @ de PV) no SSP com 22 m e de 334 kg/ha (11,1 @ de PV) no SSP com 12 m, reduções de 14,5 e de 37,8%. O maior ganho nas áreas em pleno sol deve-se à maior disponibilidade de forragem nesses sistemas. Segundo os autores, o SSP com espaçamentos intermediários possibilita bom desempenho animal e a renda com o eucalipto melhora a rentabilidade final e o tempo de retorno do sistema.

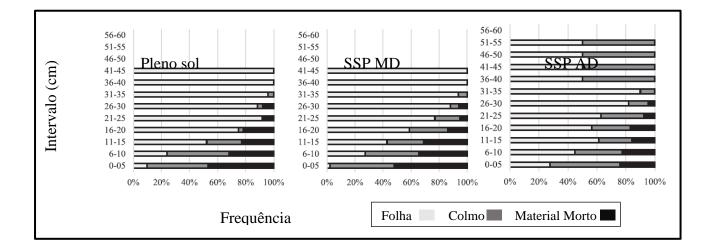
Santos et al. (2018) avaliaram o desempenho animal em pastagens de *U. brizantha* cv. Piatã em pleno sol e SSPs formados por eucalipto com 12 ou 22 m entre renques. O ganho de peso médio diário não variou entre os sistemas devido ao ajuste da oferta de forragem. Entretanto, a capacidade de suporte e a produção animal por área reduziram nos SSPs. A menor oferta de forragem e o menor ganho de peso dos animais nas áreas sombreadas também podem gerar menores receitas, o que pode prejudicar a sustentabilidade econômica desses sistemas.

Desempenho animal semelhante em pleno sol e em sistemas arborizados com a utilização de 30 m entre renques também foi observado por Magalhães et al. (2018). Já Carvalho et al. (2019) observaram maior ganho de peso por área em SSP com 37 m entre renques de

eucalipto em comparação ao pleno sol (1.194 vs. 922 kg/ha). Domiciano et al. (2020) também observaram maior ganho de peso por área em SSP com 30 m entre renques de eucalipto em comparação ao pleno sol (940 vs. 560 kg/ha). Esses resultados demonstram que, quando o objetivo do sistema é manter a máxima produção animal na área, deve-se utilizar espaçamentos sempre superiores a 30 m. Outro fator importante para manter a produção do pasto e dos animais é a realização do plantio na direção leste-oeste.

O consumo de matéria seca é um dos principais fatores que afeta o desempenho animal e está diretamente ligado à oferta de alimento, à qualidade do alimento e a variáveis dos animais, como conforto térmico e ausência de enfermidades. Em SSPs com arranjos adensados, há menor produção de forragem e, caso não se ajuste a taxa de lotação do pasto, o consumo pelos animais pode ser prejudicado. Dessa forma, é importante planejar os sistemas com maiores espaçamentos entre renques e ajustar a oferta de forragem com o objetivo de manter o adequado consumo dos animais.

Geremia et al. (2018) analisaram o comportamento ingestivo de bovinos em pastagens de *U. brizantha* cv. Piatã em pleno sol e em SSP com média densidade (eucalipto com 53 m entre renques) ou alta densidade (15 m entre renques). A altura do pasto e o intervalo de pastejo foram maiores no sistema com alta densidade. Além disso, a taxa de consumo e a massa do bocado foram menores e a taxa de bocado foi maior no sistema com alta densidade arbórea. De acordo com os autores, no pleno sol e no sistema com média densidade, o extrato superior do pasto era composto principalmente por folhas, e o inferior por colmo e material morto. Já no sistema com alta densidade, havia colmo e material morto no extrato superior do pasto (Figura 1).



**Figura 1.** Estrutura da *U. brizantha* cv. Piatã em pleno sol, em SSP com média (SSP MD) e com alta densidade (SSP AD) de acordo com a altura do pasto. Fonte: Adaptado de Geremia *et al.* (2018).

Em regiões tropicais como no Brasil, os animais criados em sistemas baseados em pastagens sofrem com as altas temperaturas e umidades, principalmente no verão. Os animais criados nessas condições sofrem os efeitos do calor e entram em estado de estresse térmico. Dessa forma, o fornecimento de sombra é uma importante estratégia para melhorar o conforto e o bem-estar animal e mitigar os efeitos negativos que o estresse calórico causa na produção.

Oliveira et al. (2019) avaliaram indicadores de conforto térmico em novilhas Nelore em um pasto controle (cinco árvores nativas) e em SSPs com 14 m entre renques ou com 22 m entre renques. As menores temperaturas do ar e em globo negro, as umidades relativas do ar e os índices de temperatura-umidade foram observadas no SSP com 14 m, no SSP com 22 m e no pleno sol, respectivamente. O melhor conforto térmico foi alcançado com a intensidade média de árvores, provavelmente devido à maior umidade no ambiente com alta densidade de árvores, o que dificulta a dissipação de calor. A temperatura vaginal aumentou duas horas depois do aumento da temperatura em globo negro. Portanto, as alterações nos ambientes sombreados nem sempre são acompanhados de melhoria no conforto térmico. Pezzopane et al. (2019) avaliaram o conforto térmico em um SSP com árvores nativas com 17 m entre renques e orientação norte-sul, em um SSP com eucalipto com 15 m entre renques, e orientação leste-oeste e no pleno sol. O número de horas com índice de temperatura em globo negro e umidade acima de 79 (valor em que o espectro indica estresse térmico) foi menor no SSP com árvores nativas (2,9 h) em comparação com o SSP com eucalipto (4,6 h) e ao pleno sol (5,2 h). Comportamento semelhante foi encontrado para a carga térmica radiante. O melhor conforto térmico no sistema com árvores nativas foi associado à orientação de plantio e à morfologia das árvores. O plantio no sentido norte-sul gerou mais áreas de sombra sob as árvores e no meio do renque em comparação ao plantio no sentido leste-oeste. Além disso, as árvores nativas apresentaram copas mais abertas e maiores e geraram mais sombra.

Giro et al. (2019) não encontraram diferença no índice de temperatura e umidade entre um SSP e o pleno sol. Porém, o índice de temperatura e umidade foi maior que 74 (desconforto térmico) apenas em janeiro e março. O índice de temperatura em globo negro e umidade foi menor na área sombreada, principalmente pela manhã. Esse fato pode indicar menor exposição ao estresse térmico, menor ganho de calor exógeno e menor gasto de energia para o processo de termólise. No entanto, segundo os autores, a pouca diferença entre os tratamentos se deve ao arranjo adotado com baixa densidade e à movimentação diária e sazonal do sol, que reduziram a formação de sombra concentrada em locais específicos. Outro aspecto relevante citado pelos autores refere-se à maior umidade e à menor velocidade dos ventos em áreas com SSP. Esses fatores, juntos, podem dificultar a perda de calor do animal para o ambiente.

### **1.5. DESEMPENHO DE BOVINOS DE LEITE EM SISTEMAS SILVIPASTORIS**

O melhor valor nutritivo do pasto e melhor ambiente nos SSPs podem aumentar a produção de leite das vacas (Paciullo et al., 2014; Améndola et al., 2019). A falta ou a inadequada oferta de sombra gera desconforto térmico e influencia negativamente o consumo de matéria seca, a produção de leite, a capacidade reprodutiva e o sistema imune (Martins et al.,

2020; Lopes et al., 2022). Entretanto, a menor produção forrageira reduz a capacidade de suporte do pasto e pode diminuir a produção total por área em sistemas com alta densidade arbórea. Assim, oferecer sombra e ambiente adequado aos animais, maximizar a produção individual e não reduzir, ou reduzir pouco, a produção do pasto e a de leite por área é o principal objetivo no planejamento desses sistemas.

Paciullo et al. (2014) estudaram, por três anos, a produção de leite de vacas F1 Holandês x Zebu e a capacidade de suporte do SSP formado por pasto de *U. decumbens, Stylosanthes* spp., *Pueraria phaseoloides* e *Calopogonium mucunoides* e um total de 70 árvores/ha de *Acacia mangium, Gliricidia sepium* e *Leucaena leucocephala* e o pleno sol formado por *U. decumbens* e *Stylosanthes* spp. A capacidade de suporte dos pastos não variou entre os sistemas, e a produção de leite das vacas foi maior no SSP no primeiro ano (10,4 kg/vaca/dia) em comparação com as vacas no pleno sol (9,5 kg/vaca/dia) e não diferiu nos anos seguintes.

Bottini-Luzardo et al. (2016) avaliaram a produção de leite de vacas criadas em monocultivo de grama-estrela (*Cynodon nlemfuensis*) ou em SSPs com grama-estrela e plantas jovens de *Leucena leucocephala* (36.000 plantas/ha). Os autores observaram produções semelhantes de leite (13,5 kg/vaca/dia no SSP e 14,5 kg/vaca/dia no pleno sol) nas vacas nos dois sistemas e maior nitrogênio ureico no leite das vacas em SSP (19,1 mg/dL nas vacas em SSP e 15,3 mg/dL naquelas em pleno sol). Os autores ressaltaram a necessidade de estabelecer estratégias para reduzir a ineficiência do uso de nitrogênio no rúmen. Possíveis estratégias para mitigar esse problema é diminuir o número de plantas de leucena por hectare, limitar o acesso dos animais aos pastos com leucena, sincronizar a degradação ruminal de carboidratos e proteína e fornecer o alimento concentrado mais vezes ao dia em menores porções.

Essa similaridade entre os tratamentos demonstra que, quando planejados de forma adequada com menor densidade arbórea, os SSPs podem não prejudicar de modo acentuado a

produção dos pastos e manter a produção animal nessas áreas. Em tais condições, o fornecimento de sombra pelas árvores gera melhor ambiência para os animais e pode aumentar a rentabilidade dos sistemas por melhorar a saúde e a reprodução dos animais e gerar nova fonte de renda proveniente da madeira.

Os resultados da literatura, principalmente a mais recente, acerca da produção de leite são muito escassos. Essa escassez pode ser atribuída a fatores que dificultam a realização de pesquisas com vacas em lactação, como a dificuldade de manejo dos animais e a falta de mão de obra nos centros de pesquisa, a necessidade de longo tempo de avaliação, a necessidade de grande número de animais por tratamento para algumas variáveis e o alto custo de implementação dos sistemas arborizados.

### 1.6. EMISSÃO DE METANO ENTÉRICO POR BOVINOS EM SISTEMAS SILVIPASTORIS

Apesar da importância para produção de alimentos, a atividade pecuária é apontada como uma das causadoras das mudanças climáticas globais devido aos impactos ambientais gerados. Esse impacto ambiental está relacionado principalmente à emissão de GEE, principalmente dióxido de carbono (CO<sub>2</sub>), metano (CH<sub>4</sub>) e óxido nitroso (N<sub>2</sub>O), que resultam no aquecimento do planeta em médio e em longo prazo. É importante ressaltar que as emissões de GEE pela agropecuária são responsáveis apenas por parte das mudanças climáticas do planeta. Segundo relatório do IPCC (2019), as atividades de agricultura, de floresta e de outros usos do solo contribuíram com aproximadamente 13% das emissões de CO<sub>2</sub>, 44% das de CH<sub>4</sub> e 81% das de N<sub>2</sub>O no período de 2007 a 2016, o que representou 23% das emissões antropogênicas totais de GEE. Embora o aquecimento global não seja gerado apenas pela agropecuária, desenvolver estratégias de mitigação das emissões dos GEE pela agropecuária

O setor agrícola brasileiro emitiu 439.213 Gg de CO<sub>2</sub> equivalente (CO<sub>2</sub> eq.) em 2016, representando 34% das emissões nacionais de GEE. Nesse período, o metano entérico foi responsável por 56,5% das emissões da agropecuária (MCTIC, 2020), sendo 90% proveniente da fermentação anaeróbica dos alimentos no rúmen. O metano é uma molécula orgânica do grupo dos hidrocarbonetos formada por ligações covalentes entre um átomo de carbono e quatro átomos de hidrogênio. No rúmen, esse gás é produzido principalmente pela atividade de microrganismos do domínio Archaea.

Esses organismos usam hidrogênio molecular (H<sub>2</sub>) para reduzir o CO<sub>2</sub> em metano por meio do ciclo de Wolfe (Thauer, 2012). A reação química simplificada que representa o ciclo da formação do metano no rúmen envolve a captação de uma molécula de CO<sub>2</sub> e quatro de H<sub>2</sub>:  $CO_2 + 4 H_2 \rightarrow CH_4 + 2 H_2O$ . A atividade metanogênica leva à perda de energia pelos animais, variando de 2 a 12% do total consumido (Sejian et al., 2011; Sejian et al., 2012). Esses valores correlacionam-se com a qualidade do alimento ingerido, sendo observado menores perdas em animais consumindo alimentos com alta digestibilidade (Benaouda et al., 2019).

Em SSPs a melhoria do valor nutricional do pasto pode melhorar a fermentação ruminal e reduzir as emissões de metano. No México, Flores-Coello et al. (2023) avaliaram a emissão de metano por vacas girolando em monocultivo de *Cynodon nlemfuensis* ou em SSPs com pasto de *Megathyrsus maximus* e *Cynodon nlemfuensis* e árvores leguminosas e árvores de porte alto e observaram menores emissões de metano por kg de matéria seca consumida (18% menor) no SSP. Entretanto, avaliações de emissão de metano por vacas leiteiras no Brasil são escassos.

Em bovinos de corte no Brasil, Pontes et al. (2018) avaliaram a emissão de metano entérico por novilhos Purunã em SSPs com 238 árvores/ha de *Eucalyptus dunnii, Schinus molle* e *Grevillea robusta* e pasto de aveia preta e azevém ou em monocultivo de pastagem. Os autores não observaram diferenças nas emissões de metano por animal (164 g/animal/dia) e por kg de peso vivo (0,58 g/kg PV), provavelmente porque a pastagem apresentava bom valor nutricional

em ambos os sistemas. Frota et al. (2017) também não observaram diferenças nas emissões de metano por bovinos em SSPs com pasto de *Megathyrsus maximus* cv. Mombaça e árvores de babaçu ou em monocultivo de pastagem. Esses resultados mostram que as diferenças no valor nutricional do pasto talvez não sejam suficientes para reduzir a emissão de metano. Uma explicação para isso é a falta de ajustes nas dietas dos animais pastejando em SSPs.

### 1.7. ESTUDOS DE METANÁLISE

Os estudos de metanálise são considerados mais robustos que estudos experimentais primários porque fazem uma síntese de todos os dados publicados sobre algum assunto. Essa metodologia se inicia no planejamento do estudo com o tema que será abordado. Após essa definição é preciso caracterizar a população, a intervenção, a comparação e os resultados (PICO) (Thomas et al., 2019). A população consiste em definir qual o contexto que os pesquisadores querem pesquisar, por exemplo, sistemas produtivos de bovinos em pastagens de braquiária. A intervenção consiste em qual o tipo de tratamento os pesquisadores desejam avaliar, por exemplo, utilização de eucalipto em pastagens de braquiária. A comparação de pastagem em monocultivo ou consorciada com eucalipto. Os resultados consistem nas respostas que os pesquisadores desejam avaliar, por exemplo, massa de forragem e ganho de peso.

A seleção de artigos pode ser feita em base de dados (Ex: Embase, Cielo, Web of Science e Scopus), diretamente em sites de revistas científicas e de centros de pesquisa ou em referências de artigos. Após essa seleção inicial, os artigos são avaliados quanto ao atendimento dos critérios PICO. Os artigos que atendem a esses critérios passam pela coleta de dados para formação de bases de dados e análise estatística.

A análise de dados é feita em modelos estatísticos, normalmente mistos ou fixos. Os modelos mistos são utilizados quando os diferentes estudos apresentam grande heterogeneidade, tornando o estudo como um efeito randômico no modelo estatístico. Por outro

lado, quando os diferentes estudos são conduzidos de forma homogênea, considera-se que eles apresentam o mesmo efeito sobre as respostas e utiliza-se modelo de efeito fixo. Nesses modelos o efeito da intervenção normalmente é expresso pela diferença média bruta (RMD – raw mean difference), que expressa a diferença entre a média do grupo tratado e do não-tratado. Além disso, o efeito que cada comparação sobre a média geral é definida pelo inverso da variância, ou seja, quando menor a variância do estudo, maior a influência desse estudo sobre a média geral (Higgins et al., 2019).

### **1.8. CONSIDERAÇÕES FINAIS**

Os SSPs conciliam aumentos na produção animal e nos indicadores ambientais. Entretanto, para obter esses benefícios é preciso estabelecer um planejamento técnico criterioso durante a implementação do sistema para manter a produtividade do pasto e dos animais na área. A melhoria do valor nutricional do pasto pode reduzir as emissões de metano, porém os dados sobre emissões em rebanhos leiteiros em SSPs são escassos.

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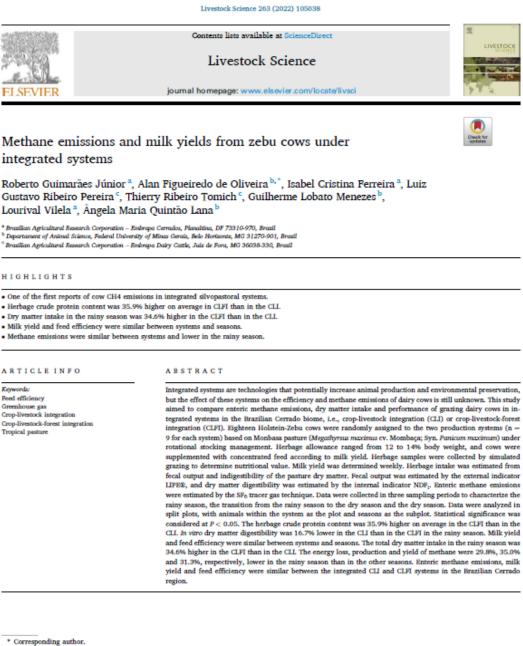
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### 1 CAPÍTULO 2: ARTIGO - METHANE EMISSIONS AND MILK YIELDS FROM

### 2 ZEBU COWS UNDER INTEGRATED SYSTEMS

### 3 Artigo publicado na Livestock Science (Impact Factor 1.929)



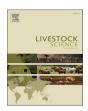
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# Methane emissions and milk yields from zebu cows under integrated systems

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#### HIGHLIGHTS

- One of the first reports of cow CH4 emissions in integrated silvopastoral systems.
- Herbage crude protein content was 35.9% higher on average in CLFI than in the CLI.
- Dry matter intake in the rainy season was 34.6% higher in the CLFI than in the CLI.
- Milk yield and feed efficiency were similar between systems and seasons.
- Methane emissions were similar between systems and lower in the rainy season.

#### ARTICLE INFO

Keywords: Feed efficiency Greenhouse gas Crop-livestock integration Crop-livestock-forest integration Tropical pasture

#### ABSTRACT

Integrated systems are technologies that potentially increase animal production and environmental preservation, but the effect of these systems on the efficiency and methane emissions of dairy cows is still unknown. This study aimed to compare enteric methane emissions, dry matter intake and performance of grazing dairy cows in integrated systems in the Brazilian Cerrado biome, i.e., crop-livestock integration (CLI) or crop-livestock-forest integration (CLFI). Eighteen Holstein-Zebu cows were randomly assigned to the two production systems (n = 9 for each system) based on Monbasa pasture (Megathyrsus maximus cv. Mombaça; Syn. Panicum maximum) under rotational stocking management. Herbage allowance ranged from 12 to 14% body weight, and cows were supplemented with concentrated feed according to milk yield. Herbage samples were collected by simulated grazing to determine nutritional value. Milk yield was determined weekly. Herbage intake was estimated from fecal output and indigestibility of the pasture dry matter. Fecal output was estimated by the external indicator LIPE®, and dry matter digestibility was estimated by the internal indicator NDF<sub>i</sub>. Enteric methane emissions were estimated by the SF<sub>6</sub> tracer gas technique. Data were collected in three sampling periods to characterize the rainy season, the transition from the rainy season to the dry season and the dry season. Data were analyzed in split plots, with animals within the system as the plot and seasons as the subplot. Statistical significance was considered at P < 0.05. The herbage crude protein content was 35.9% higher on average in the CLFI than in the CLI. In vitro dry matter digestibility was 16.7% lower in the CLI than in the CLFI in the rainy season. Milk yield and feed efficiency were similar between systems and seasons. The total dry matter intake in the rainy season was 34.6% higher in the CLFI than in the CLI. The energy loss, production and yield of methane were 29.8%, 35.0% and 31.3%, respectively, lower in the rainy season than in the other seasons. Enteric methane emissions, milk yield and feed efficiency were similar between the integrated CLI and CLFI systems in the Brazilian Cerrado region.

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#### 1. Introduction

The Cerrado biome (i.e., Brazilian Savannah) of Brazil occupies approximately 204 million hectares (24% of the national territory). Inadequate management in cattle farming, such as unsuitable stocking rates and lack of soil fertility maintenance, can lead to environmental degradation in this biome (Dias-Filho et al., 2014; Cerri et al., 2015). Briefly, integrated systems can be defined as the simultaneous cultivation, in succession or in rotation, of different plant and animal species in the same area. Therefore, integrated systems have been proposed as a strategy to promote the sustainable use of resources, reduce environmental impacts and increase agricultural productivity in this biome (Lemaire et al., 2013).

In 2016, the Brazilian agricultural sector was responsible for the emission of 439,213 Gg of CO<sub>2</sub> equivalents (CO<sub>2</sub> eq.), representing 34% of the national emissions of greenhouse gases (GHG). In the same year, enteric methane (CH<sub>4</sub>) emissions represented 56.5% of agricultural emissions (MCTIC, 2020). In the context of agricultural decarbonization in the tropics, the use of integrated systems has been identified as a promising sustainable strategy (Norse, 2012; Figueiredo et al., 2016; Torres et al., 2017). Crop-livestock integration (CLI) and crop-livestock-forest integration (CLFI) are the two forms of integration. CLI is defined as the integration of different crops and animals, whereas CLFI is defined as the integration of crops, animals and forestry. Both integrations are implemented in the same area to explore possible synergism among the components, which would increase system productivity and income outputs (Paciullo et al., 2014; Magalhães et al., 2018; Oliveira et al., 2022). Among the main benefits of this system are greater carbon stock (Almeida et al., 2021), better animal comfort and welfare (Martins et al., 2021; Reis et al., 2021), better herbage nutritional value (Lima et al., 2018) and farm income diversification (Müller et al., 2011). Additionally, according to Liu et al. (2021), by continuously improving production efficiency, livestock can be a short-term solution to mitigate anthropogenic effects on climate change while long-term solutions for carbon emissions from fossil fuel use are developed.

According to Silva et al. (2013), intake by grazing cattle is primarily influenced by sward structure and secondarily by nutritional value. Geremia et al. (2013) showed that silvopastoral systems (SPSs) with moderate shading (49 m between ranks; 338 trees  $ha^{-1}$ ) provided an intake rate, bite mass and bite rate similar to those of pasture monoculture. In these SPSs with moderate shade, improved herbage nutritional value, especially the increase in protein content (Paciullo et al., 2014; Geremia et al., 2018; Santos et al., 2018), and better thermal comfort during the day (Giro et al., 2019; Martins et al., 2021) can increase intake and improve feed efficiency and cattle performance (Santos et al., 2018). The greater efficiency of nutrient utilization by dairy cows can increase the overall efficiency of the production system (Lemaire et al., 2013; Soussana and Lemaire, 2014). Furthermore, improved comfort caused by shading can also reduce energy use for controlling thermal stress and increase animal efficiency (Schütz et al., 2010; Vizzotto et al., 2015), especially in tropical conditions (Reis et al., 2021).

The main GHG generated in ruminant production systems is enteric  $CH_4$  (Hagemann et al., 2011; O'Brien et al., 2012; Yan et al., 2013). In SPSs,  $CH_4$  emissions can be reduced by improving pasture nutritional value (Pedreira et al., 2009). However, an integrated assessment of animal production and GHG emissions to better characterize the efficiency and sustainability of these animal production systems is lacking. Furthermore, the determination of  $CH_4$  emission factors that are specific to these systems must be developed to improve the accuracy of the GHG emissions inventory.

This study aimed to compare enteric  $CH_4$  emissions, dry matter intake (DMI), milk yield (MY) and feed efficiency of grazing dairy cows in two integrated systems, CLI vs. CLFI, both of which are typical of the Brazilian Cerrado biome. The study spanned the rainy and dry seasons as well as the rainy-to-dry transition. Our first hypothesis was that, regardless of the seasons, the CLFI system would have improved pasture nutritional value compared to that in the CLI system, and this improvement would result in increased feed intake and feed efficiency. Our second hypothesis was that higher DMI increases enteric CH<sub>4</sub> production (in g/day) but reduces CH<sub>4</sub> yield (in g CH<sub>4</sub>/kg DMI) and intensity (in g CH<sub>4</sub>/kg MY) in dairy cows.

#### 2. Materials and methods

Experimental procedures were approved by the animal use ethics committee of Embrapa Cerrados (protocol  $n^{0}$ . 533-2541-1/2017).

#### 2.1. Experimental area and treatments

The study was carried out in the Cerrado biome at the Center of Technology for Dairy Zebu Breeds, located in Brasília, DF, Brazil (15°57′09" S, 48°08′12" W, altitude 998 m). The climate is classified as tropical rainy Awa (A - tropical rainy climate, w - rainy summer, a - hot summer, with average temperature of the hottest month above 22 °C) (Alvares et al., 2013). The Cerrado biome has two well-defined climatic seasons with hot and rainy summers (rainy season; between October and March) and cold and rainless winters (dry season; between April and September). The experimental area's soil is characterized as red ferral-sols (WRB, 2006).

The treatments consisted of integrated production systems based on Mombaça grass (*Megathyrsus maximus* Syn. *Panicum maximum* cv. Mombaça) established in succession with soybeans (*Glycine max*) in the CLI and CLFI. Trees in the CLFI were planted in an east–west orientation in 2013 with simple rows of *Eucalyptus urograndis* spaced 25 m apart with a density of 130 trees/ha (which can be considered low density), and pasture was implemented in 2016. At the evaluation times, the trees were approximately 28 m tall. The experiment lasted 95 days from February to May 2019 and comprised three sampling periods as follows: rainy (February), transition (March), and dry (May) seasons.

The soil chemical characteristics in the 0–20 cm layer in the CLFI were pH = 6.2, soil organic matter (SOM) = 33.7 g dm<sup>-3</sup>, P = 14.1 mg dm<sup>-3</sup>, K = 202 mg dm<sup>-3</sup>, Ca = 1.1 cmol dm<sup>-3</sup>, Mg = 0.7 cmol dm<sup>-3</sup>, Al = 0.01 cmol dm<sup>-3</sup>, and H + Al =1.7 cmol dm<sup>-3</sup>; those in the CLI were pH = 6.1, SOM = 26.6 g dm<sup>-3</sup>, P = 18.91 mg dm<sup>-3</sup>, K = 140 mg dm<sup>-3</sup>, Ca = 2.1 cmol dm<sup>-3</sup>, Mg = 0.6 cmol dm<sup>-3</sup>, Al = 0.01 cmol dm<sup>-3</sup>, and H + Al = 1.8 cmol dm<sup>-3</sup>. The pasture area was fertilized with urea during the experimental period with two applications of 54 kg ha<sup>-1</sup> (totaling 108 kg N ha<sup>-1</sup>) in the CLI and in the CLFI.

#### 2.2. Animal management

Eighteen lactating Holstein-Zebu cows were used as repetitions (test animals). The animals were evenly distributed considering days in milk (DIM), MY and body weight (BW), with nine cows in the CLI (MY = 16.5  $\pm$  4.28 kg/cow.day, DIM = 95.2  $\pm$  49.4 days and BW = 490  $\pm$  51.4 kg) and nine in the CLFI (MY = 18.9  $\pm$  4.74 kg/cow.day, DIM = 98.7  $\pm$  42.8 days and BW 498  $\pm$  72.9 kg). The CLI and CLFI areas contained 8 ha each. Each of these systems' areas was divided into 12 paddocks and managed in rotational grazing with a variable stocking rate, with two to three grazing days and 22 or 33 rest days in the rainy and dry seasons, respectively, to maintain an average herbage allowance of 12–14 kg of dry matter (DM) per 100 kg of BW, according to herbage mass evaluations.

Cows received concentrated feed based on corn (*Zea mays*) and soybeans (180 g/kg of crude protein and 760 g/kg of total digestible nutrients) with the proportion of one kg for every three kg of milk produced (based on individual yield) when cows produced more than eight kg of milk per day. Concentrate was offered during the morning and afternoon milkings. In addition, cows received a water and mineral mixture (80 g/kg phosphorus, 115 g/kg sodium, 30 mg/kg selenium and 3000 mg/kg zinc) *ad libitum*.

#### 2.3. Herbage chemical composition

Herbage samples were manually collected from paddocks during grazing days in both systems during the three seasons. Sampling was carried out by simulated grazing to represent pasture strata grazed by the animals (Aroeira et al., 1999). Samples were dried in an oven at 55 °C for 72 h and processed in a knife mill with 1 mm sieves (Thomas Wiley Model 4, Thomas Scientific, Swedesboro, NJ, USA). Crude protein contents (Method 976.05; AOAC, 1990) were determined by the Kjeldahl method. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined according to Van Soest et al. (1991) in an Ankom fiber analyzer (Ankom Technology, Macedon, NY, USA) with methodology adapted by ANKOM (2021). Neutral detergent fiber residues were submitted to CP analysis to determine neutral detergent insoluble protein (NDIP). In vitro dry matter digestibility (IVDMD) was determined by the methodology proposed by Tilley and Terry (1963) with adaptations for execution in the Daisy<sup>II</sup> digestion apparatus (Ankom Technology, Macedon, NY, USA) described by Mabjeesh et al. (2000).

#### 2.4. Milk yield and feed intake

Milk yield was determined weekly during the experiment. On the day of MY evaluation, individual samples were collected to determine milk fat content. Milk yield was corrected to 4% fat (4% FCM) according to the equation proposed by Gaines (1928):

 $4\% FCM = (0.4 \ x \ MY) + [15 \ x \ (MFY \ x \ MY \ / \ 100)]$ 

where 4% FCM = 4% fat corrected milk yield (kg/cow.day), MY = milk yield (kg/cow.day), and MFY = milk fat yield (kg/cow.day).

Fecal output was estimated using the external indicator LIPE® (isolated, purified and enriched *Eucalyptus grandis* lignin) (Berchielli et al., 2000; Saliba, 2005), and dry matter digestibility was estimated by the internal indicator indigestible neutral detergent fiber (NDF<sub>i</sub>) (Casali et al., 2008). The external indicator LIPE® was offered in capsules at a dose of 500 mg per cow/day for six consecutive days in each season (rainy season, transition season and dry season) (Saliba et al., 2013). The protocol used three days of adaptation to the indicator followed by three days of feces collection, carried out directly in rectal ampoules.

Fecal samples were collected twice a day after milking. Samples were dried in an oven at 55 °C for 72 hs and processed in a Wiley knife mill with 1 mm sieves (Thomas Wiley Model 4, Thomas Scientific, Swedesboro, NJ, EUA). Equal amounts of each sample from each collection were used to form composite samples of each animal by season. Approximately 10 g of each composite sample was used to determine the LIPE® concentration by infrared spectroscopy in a spectrophotometer (Varian 800 FT-IR, Varian Systems - Inc, Palo Alto, CA, USA) with Fourier transform (FT-IR) (Saliba, 2005; Saliba et al., 2013). Fecal output (FO) was estimated by the equation:

#### $FO = (ingested \ dose \ of \ LIPE^{(\mbox{\tiny R})} \ / \ Fecal \ concentration \ of \ LIPE^{(\mbox{\tiny R})})$

For NDF<sub>i</sub> determination, 0.8 g of feces and herbage samples were weighed into F57 bags (Ankom Technology, Macedon, NY, USA) in triplicate and incubated for 264 h in crossbred steer (3/4 Holstein x Gyr) (Casali et al., 2008). After incubation, F57 bags were washed in water and submitted to NDF analysis according to Van Soest et al. (1991) in an Ankom fiber analyzer (Ankom Technology, Macedon, NY, USA) with methodology adapted by ANKOM (2021). Dry matter digestibility (DIG) was determined using the equation:

$$DIG = [1 - (NDFip / NDFif)]$$

where NDFip = indigestible neutral detergent fiber from herbage and NDFif = indigestible neutral detergent fiber from feces.

Individual intake of herbage and concentrate were determined by the equation:

DMI = [FO / (1 - DIG)]

where DMI = dry matter intake (kg/cow.day), FO = fecal output (kg/cow.day), and DIG = dry matter digestibility (% DM).

Total dry matter intake (TDMI) was determined as the sum of herbage and concentrate intake. Herbage intake, concentrate and TDMI were expressed as % BW. The feed efficiency (FE) was determined by the equation:

$$FE = (4\% FCM / TDMI)$$

where FE = feed efficiency; 4% FCM = 4% fat corrected milk yield (kg/ cow.day); and TDMI = total dry matter intake (kg/cow.day).

#### 2.5. Methane emission

The CH<sub>4</sub> emission was estimated using the sulfur hexafluoride trace gas dilution technique (SF<sub>6</sub>) (Johnson et al., 1994) for at least four consecutive days per animal in each season (rainy season, transition season and dry season). One cow from the CLI treatment was excluded from this assessment due to its low daily rate of SF<sub>6</sub> capsule emission. Regarding CH<sub>4</sub> emissions, the variables calculated were ruminal CH<sub>4</sub> production (g CH<sub>4</sub>/day), intensity (g CH<sub>4</sub>/4%FCM.day) and yield (g CH<sub>4</sub>/kg DM). These parameters were estimated by the following equations:

MP = [(CAF \* (MCA - MCC)) / (SCA - SCC)] \* 60 \* 24

where MP = ruminal CH<sub>4</sub> production (g CH<sub>4</sub>/day), CAF = capsule average flow (g/min), MCA = CH<sub>4</sub> concentration in the animal's yoke ( $\mu$ g/m<sup>3</sup>), MCC = CH<sub>4</sub> concentration in the control's yoke ( $\mu$ g/m<sup>3</sup>), SCA = SF<sub>6</sub> concentration in the animal's yoke ( $\mu$ g/m<sup>3</sup>), and SCC = SF<sub>6</sub> concentration in the control's yoke ( $\mu$ g/m<sup>3</sup>).

$$MEI = (MP / 4\% FCM)$$

where  $MEI = CH_4$  emission intensity (g  $CH_4/4\%$ FCM.day), MP = ruminal CH<sub>4</sub> production (g  $CH_4/day$ ), and 4%FCM = 4% fat corrected milk yield (kg/cow.day).

$$MEY = (MP / TDMI)$$

where  $MEY = CH_4$  yield (g  $CH_4$ /kg DM), MP = ruminal  $CH_4$  production (g  $CH_4$ /day), and TDMI = total dry matter intake (kg/cow.day).

After determination of the individual herbage and concentrate intake, herbage and concentrate samples were submitted to combustion in an adiabatic calorimetric pump (Model PARR 2081 - PARR Instrument Company, Moline, IL, USA) to determine feed gross energy. The herbage gross energy intake was determined by multiplying the gross energy and individual herbage intake, the concentrate gross energy intake was determined by multiplying the gross energy and individual concentrate intake, and the total energy intake was determined by adding the herbage and concentrate gross energy intake. The gross energy loss as  $CH_4$  (Ym, %) was estimated by the equation:

$$Ym = [(MP * 13334) / GDEI) * 100]$$

where Ym (%) = gross energy loss as  $CH_4$  (%), MP = ruminal  $CH_4$  production (g  $CH_4$ /day), 13334 =  $CH_4$  gross energy concentration (cal/g), and GDEI = gross dietary energy intake (cal/cow.day).

#### 2.6. Statistical analyses

Data were submitted to Shapiro–Wilk's and Barttlet's tests to verify the assumptions of normality and variance homogeneity, respectively. However, no variable needed to be transformed. Data were analyzed by analysis of variance (2-way ANOVA) using a split-plot arrangement with repeated measures over time, with "animals within system" as the plot and "seasons" as the subplot. Production system, season and their interaction were considered fixed effects, and animals were considered random effects. As repeated measures over time are not totally independent (nonzero covariation), Mauchly's test (Mauchly, 1940) was applied to check whether there was a need to correct the analysis of variance. When Mauchly's test was significant (P < 0.05), a correction was performed using Greenhouse–Geisser's test (Greenhouse and Geisser, 1959).

Days in milk was tested as a covariate for all of the variables measured in the animals and was incorporated into the model for variables for which DIM had a significant effect (P < 0.05). Season means were compared by Tukey's test and systems by Fisher's test (P < 0.05). Pearson's correlation analysis was performed between variables (P < 0.05). Correlation was considered weak when the correlation coefficient was less than 30%, moderate when the correlation coefficient was greater than 70%. All analyses were performed in the R Core Team (2019) software.

#### 3. Results

The herbage CP content showed a significant interaction between system and season (P = 0.007) (Table 1). The crude protein content in the CLFI was similar between seasons, but in the CLI, it was 29.3% lower in the dry season than in the other seasons. Crude protein was similar between systems in the transition season, but it was 31.4% and 83.0% higher in the CLFI than in the CLI in the rainy and dry seasons, respectively. Neutral detergent fiber and ADL were not influenced by any evaluated factor (P > 0.05). Acid detergent fiber was 7.50% higher (P = 0.05) in the CLI than in the CLFI and 11.3% lower (P = 0.004) in the dry season than in the other seasons.

In vitro dry matter digestibility showed a significant interaction between system and season (P = 0.028). In vitro dry matter digestibility in the CLFI was similar among seasons, but in the CLI, it was lower in the rainy season than in the other seasons. In the transition and dry seasons, IVDMD was similar between systems, but in the rainy season, IVDMD was 16.7% lower in the CLI than in the CLFI. Neutral detergent insoluble protein was 21.7% higher (P = 0.034) in the CLI than in the CLFI. Neutral detergent insoluble protein was lower (P = 0.013) in the rainy season, intermediate in the transition season and higher in the dry season.

Milk yield, 4% FCM and milk fat content were not altered by any evaluated factor (P > 0.05) (Table 2). Concentrate intake was 24.6% lower (P = 0.009) in the dry season than in the other seasons (Table 3). Herbage intake showed a significant interaction between system and season (P = 0.002). Herbage intake in the CLFI was 55.7% lower in the dry season than in other seasons, but in the CLI, it was lower in the rainy season, intermediate in the dry season and higher in the transition season. Total dry matter intake showed a significant interaction (P = 0.003) between system and season. The total dry matter intake in the CLFI was 50.5% lower in the dry season than in the other seasons. The total dry matter intake in the CLFI was 50.5% lower in the dry season than in the other seasons. The total dry matter intake in the transition station than at the other stations. The total dry matter intake in the rainy season was 34.6% higher in the CLFI than in the CLI, with no differences in the other seasons. Feed efficiency showed an interaction between system and season (P = 0.045).

The gross energy losses of CH<sub>4</sub>, CH<sub>4</sub> production, and CH<sub>4</sub> yield were 29.8%, 35.0% and 31.3% lower (P < 0.01), respectively, in the rainy season than in the other seasons (Table 4). Milk yield corrected to 4% fat showed a moderate positive correlation with concentrate intake and a negative correlation with CH<sub>4</sub> emissions (Fig. 1). Milk yield corrected to 4% fat showed a strong positive correlation with feed efficiency. Concentrate intake showed a moderate positive correlation with TDMI and a negative correlation with CH<sub>4</sub> emissions. Herbage intake was strongly positively correlated with TDMI and moderately negatively correlated with feed efficiency. Total dry matter intake showed a moderate negative correlation with CH<sub>4</sub> emissions.

#### 4. Discussion

#### 4.1. Nutritional value

The increase in herbage CP content in SPSs compared to full sun is a result that has been described in previous studies with tropical grasses under shade (Geremia et al., 2018; Lima et al., 2018; Santos et al., 2018; Oliveira et al., 2022). This increase is mainly due to physiological changes in plants in SPSs that allow plant cells to remain younger

Table 1

Crude protein, neutral detergent fiber, acid detergent fiber, acid detergent lignin, *in vitro* dry matter digestibility and neutral detergent insoluble protein (DM basis) of *Megathyrsus maximum* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil.

System	Season			Mean	SEM	P-valueS	P-valueSE	P value S * SE
	Rainy	Transition	Dry					
Crude protein	(g/kg DM)							
CLFI	116aA	135aA	136aA	-	6.2	< 0.001	< 0.001	0.007
CLI	88.3aB	122aA	74.3bB	-				
Neutral deter	gent fiber (g/kg DM)	)						
CLFI	623	673	615	-	8.8	0.400	0.264	0.105
CLI	679	643	635	-				
Acid detergen	t fiber (g/kg DM)							
CLFI	322	322	278	307B	6.3	0.050	0.004	0.420
CLI	356	328	305	330A				
Mean	339a	324a	294b					
Acid detergen	t lignin (g/kg DM)							
CLFI	43.4	57.1	54.3	-	2.05	0.217	0.619	0.087
CLI	49.4	42.5	45.7	-				
In vitro dry n	atter digestibility (g	/kg DM)						
CLFI	651aA	648aA	666aA	-	12.0	0.005	0.028	0.028
CLI	542bB	650aA	626aA	-				
Neutral deter	gent insoluble proteir	n (g/kg DM)						
CLFI	35.1	36.9	45.6	39.2B	2.45	0.034	0.013	0.295
CLI	35.8	50.8	56.6	47.7A				
Mean	35.4b	42.9ab	52.2a					

Means followed by different lowercase letters in the line differ by the Tukey' test and uppercase letter in the column differ by the Fisher' test. CLFI, crop-livestockforestry integration; CLI, crop-livestock integration; SEM, standard error of mean; *P*-value S, *P* value for system effect; *P*-value SE, *P* value for season effect; *P*-value S \* SE, *P* value for interaction between system and season effect.

#### Table 2

Milk yield, 4% fat corrected milk yield and milk fat of crossbred dairy cows grazing *Megathyrsus maximum* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil.

System	Season	Season			P-valueCOV	P-valueS	P-valueSE	P-valueS * SE
	Rainy	Transition	Dry					
Milk yield (kg	/cow.day)							
CLFI	18.9	16.9	13.1	0.71	< 0.001	0.135	0.207	0.139
CLI	16.6	17.6	13.4					
4% fat correct	ted milk yield (kg/c	ow.day)						
CLFI	19.5	17.8	13.9	0.68	< 0.001	0.085	0.574	0.099
CLI	16.6	18.3	13.9					
Milk fat (%)								
CLFI	4.30	4.50	4.60	0.102	0.092	0.517	0.300	0.995#
CLI	4.10	4.30	4.30					

Means followed by different lowercase letters in the line differ by the Tukey' test. CLFI, crop-livestock-forestry integration; CLI, crop-livestock integration; SEM, standard error of mean; *P*-value COV, *P*-value for covariate days in milk, *P*-value S, *P* value for system effect; *P*-value SE, P value for season effect; *P*-value S \* SE, *P* value for interaction between system and season effect; *#*, *P* value corrected for Greenhouse-Geisser.

#### Table 3

Concentrate intake, herbage intake, total dry matter intake and feed efficiency of crossbred dairy cows grazing *Megathyrsus maximus* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil.

System	Season			SEM	P-valueCOV	P-value S	P-value SE	P-valueS * SE
	Rainy	Transition	Dry					
Concentrate d	lry matter intake (%	BW)						
CLFI	0.883	0.830	0.680	0.0321	0.180	0.854	0.009	0.367
CLI	0.897	0.800	0.606					
Mean	0.890a	0.816a	0.643b					
Herbage dry 1	natter intake (% BW	Ŋ						
CLFI	1.90aA	2.21aA	1.32bA	0.068	0.099	0.122	< 0.001	0.002
CLI	1.51cB	2.05aA	1.67bA					
Total dry mat	ter intake (% BW)							
CLFI	2.80aA	2.97aA	1.92bA	0.078	0.321	0.229	0.002#	0.003
CLI	2.08bB	2.83aA	2.34bA					
Feed efficienc	y (kg milk/kg DM)							
CLFI	1.40aA	1.21aA	1.41aA	0.061	< 0.001	0.146	0.038	0.045
CLI	1.66aA	1.31aA	1.12aA					

Means followed by different lowercase letters in the line differ by the Tukey' test and uppercase letter in the column differ by the Fisher' test. CLFI, crop-livestockforestry integration; CLI, crop-livestock integration; SEM, standard error of mean; *P*-value COV, *P*-value for covariate days in milk, *P*-value *S*, *P* value for system effect; *P*-value SE, P value for season effect; *P*-value S \* SE, P value for interaction between system and season effect; DM, dry matter; #, P value corrected for Greenhouse-Geisser.

#### Table 4

Enteric CH<sub>4</sub> emissions and gross energy loss as enteric CH<sub>4</sub> of crossbred dairy cows grazing *Megathyrsus maximus* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil.

System	Season			SEM	P-value COV	P-valueS	P-value SE	P-valueS * SE
	Rainy	Transition	Dry					
Methane prod	luction (g CH4/day)							
CLFI	351	500	451	23.3	0.465	0.743	< 0.001	0.389#
CLI	297	583	471					
Mean	325b	541a	460a					
Emission inter	nsity (g CH <sub>4</sub> /4%FC	M.day)						
CLFI	18.5	31.5	32.9	1.68	0.013	0.761	0.087	0.943
CLI	19.8	32.0	35.3					
Methane yield	l (g CH₄/kg DM)							
CLFI	25.2	34.2	44.4	1.81	0.612	0.512	0.006	0.310#
CLI	29.5	41.2	38.5					
Mean	27.2b	37.7a	41.5a					
Ym (%)								
CLFI	8.33	10.9	13.9	0.58	0.642	0.387	0.009	0.327#
CLI	9.63	13.6	12.4					
Mean	8.94b	12.3a	13.2a					

Means followed by different lowercase letters in the line differ by the Tukey' test and uppercase letter in the column differ by the Fisher' test. CLFI, crop-livestock-forestry integration; CLI, crop-livestock integration; SEM, standard error of mean; #, *p* value corrected for Greenhouse-Geisser; CH<sub>4</sub>, methane; DM, dry matter; FCM, 4% fat corrected milk, Ym, gross energy loss as CH<sub>4</sub> (% of ingested). *P*-value COV, *P*-value for covariate days in milk, *P*-value S, *P* value for system effect; *P*-value SE, *P* value for interaction between system and season effect.

(Guenni et al., 2008; Taiz et al., 2015; Guenni et al., 2018), accumulate fewer fibrous compounds (Geremia et al., 2018; Lima et al., 2018) and proportionally have higher CP. Another factor that may explain the CP

increase is the greater soil nitrogen availability (Wilson, 1996; Chatterjee et al., 2018). The more uniform CP content in the CLFI between seasons is due to the lower metabolic stress of plant cells in the CLFI

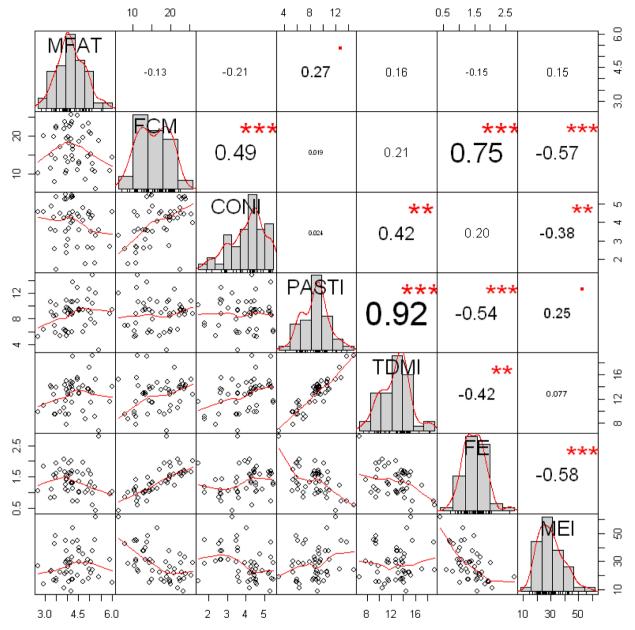


Fig. 1. Matrix of correlation between performance, feed intake and methane emission of crossbred cows grazing *Megathyrsus maximus* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil. MFAT, milk fat; FCM, 4% fat corrected milk yield; CONI, concentrate intake; PASTI, pasture intake; TDMI, total dry matter intake; FE, feed efficiency; MEI, methane emission intensity; values inside the box indicate the coefficient of correlation; \*\*\* = P-value < 0.001; \*\* = P-value < 0.01; \* = P-value < 0.05.

compared to the CLI. Lower exposures to UV-B radiation, extreme temperatures and intense light delay the senescence process (Gómez et al., 2012; Taiz et al., 2015; Santiago-Hernández et al., 2016) of plant cells under shade, which explains the CP content maintenance in the CLFI.

Increases in height and stem percentage in plants are factors that can increase NDF. However, the maintenance of cells at a younger stage and a lower senescent material percentage can reduce NDF. Therefore, these factors together explain the equality of NDF between systems (Paciullo et al., 2014; Geremia et al., 2018; Silva et al., 2020). Although NDF and ADL were similar between systems, ADF was higher in the CLI, which may have generated higher IVDMD in the CLFI in the rainy season. This higher herbage IVDMD probably also occurred due to higher CP and lower NDIP contents. These results confirm the hypothesis that CLFI improves herbage nutritional value and indicate that, in the Cerrado region, pastures cultivated in CLFI systems can offer better quality

herbage for animals, especially in the rainy season.

Higher NDIP contents in herbage under full sun were also observed by Paciullo et al. (2016), who found 14% lower NDIP in *Panicum* cultivars subjected to 58% shading. These results are important because they indicate that herbage plant cells in CLFIs show fewer chemical bonds between fibrous and protein compounds, which probably increase IVDMD and may increase nutrient supply to animals (Van Soest et al., 1994).

#### 4.2. Performance and feed intake

Although herbage had better nutritional value in the CLFI and in the rainy season, cows had similar MY. Martins et al. (2021) and Paciullo et al. (2014) also did not observe any effect of the silvopastoral system on the MY of Holstein-Zebu cows in the Cerrado and Atlantic Forest biomes in Brazil, respectively. These results probably occurred because

the animals received concentrate supplementation according to MY, which supplied the nutrients that were deficient in the pasture.

Supplementation with concentrated feed is a management practice normally adopted on farms that produce milk from grazing animals in Brazil. Bottini-Luzardo et al. (2016) also did not observe a difference in the MY of cows in SPSs with *Leucaena leucocephala* and *Cynodon nlemfuensis* compared to full sun. These authors observed greater blood urea nitrogen of cows in SPSs compared to full sun (19.1 vs. 15.3 mg/dL), probably due to the failure in synchronism between ruminal metabolism of protein and carbohydrates.

Bretas et al. (2020) observed higher nitrogen concentrations in the excreta of animals in a CLFI compared to those in full sun, which corroborates the hypothesis of lower efficiency in protein utilization. This failure in synchronism occurs due to the rapid availability of nonprotein herbage nitrogen fractions in the rumen, and this excess nitrogen is excreted as urea (Kolver et al., 1998; Zhang et al., 2020). Milk yield equality of animals grazing herbage with higher CP content in the CLFI may indicate that in commercial farms, the balancing of diets could use lower CP content in concentrated feed and reduce nutrition costs. Therefore, future studies should evaluate different concentrations of protein supplementation for dairy cows in CLFIs, which may indicate greater production efficiency with lower protein supplements.

Another factor that may have generated similar MY between systems is the cows' lactation stage. The cows had already passed the lactation peak, and at this stage, these animals have low productive efficiency because they change their energy metabolism to produce body tissues (Santos et al., 2014; Lage et al., 2021). In addition, cows had medium MY and therefore did not have a very high demand for nutrients. Under these conditions, supplementation with concentrated feed probably met the cows' requirements in the CLI, and there was no limitation of protein and amino acids. This adequate supply of nutrients allowed for similar yields to cows in the CLFI, even though they were consuming a diet with lower CP content. Furthermore, the cows' body weights were not changed during the experiment, which indicates that the animals in the present study had no feed restriction.

As concentrated feed was supplied according to MY, the animals with higher yield also ingested more concentrate, which explains the correlation between 4% FCM and concentrate intake. This result was corroborated by concentrate intake, which was also lower in the dry season than in the other sampling periods. Furthermore, the results showed that the most productive animals were also more efficient and emitted less CH<sub>4</sub>. Britt et al. (2003) also observed a positive correlation between feed efficiency and MY (r = 66.4; P < 0.001). These results indicate the need to select animals with high productive capacity and lower dry matter intake to increase the productivity efficiency of dairy cows and reduce the environmental impact (Yan et al., 2013) of integrated systems in the Cerrado region.

Herbage intake was higher in the CLFI than the CLI in the rainy season, which also increased total dry matter intake. Wims et al. (2010) also observed higher herbage intake by dairy cows (16.9 vs. 15.4 kg DM/cow.day) in pastures of better quality compared to those in pastures of lower quality. This higher intake occurred due to better herbage nutritional value demonstrated by higher CP content, higher IVDMD and lower NDIP. The intake of grazing cows is mainly influenced by a physical limitation caused by ruminal filling (Allen, 1996; Mertens and Grant, 2020). Therefore, the higher herbage IVDMD and lower NDIP in the CLFI may have increased the flow of digesta through the gastrointestinal tract, reduced physical limitation and increased the intake of cows in the rainy season. In addition, higher temperature and solar radiation in the CLI in the rainy season likely reduced cow comfort and may have reduced grazing time and herbage intake (Karvatte-Júnior et al., 2016; Oliveira et al., 2017; Pezzopane et al., 2019).

Herbage intake in the CLFI was lower in the dry season, probably due to a reduction in nutritional value. In addition, worse herbage structure in the dry period may cause reduced intake (Santos et al., 2016; Santos et al., 2018; Nascimento et al., 2021). Geremia et al. (2018) observed

lower bite mass (1.00 vs. 1.20 g DM/bite) and intake rate (45.9 vs. 49.2 g DM/min) in dairy heifers on CLFIs in the rainy season compared to those in the dry season. These results corroborate the lower intake observed in the dry season in the present study due to worse herbage structure.

Although not evaluated in the present study, heat stress probably reduced herbage intake in the rainy season. Similar to the present study, Martins et al. (2021) observed a lower black globe temperature and humidity index (82.4 vs. 88.9), udder temperature (35.3 vs. 37.1°C) and eye temperature (35.4 vs. 36.4°C) in the CLFI than in the CLI, which indicates better animal thermal comfort in the CLFI and may have improved herbage intake. National Research Council (2001) also emphasizes the negative effect of heat stress on intake. In addition, as cows were producing more milk in the rainy season, concentrate intake was also higher and may have reduced replacement herbage intake.

As concentrate intake was not influenced by the systems, the highest TDMI observed in the CLFI in the rainy season was due to higher herbage intake. Sousa et al. (2008) and Santos et al. (2012) observed a TDMI of 2.5 and 2.39% BW, respectively, in Girolando cows grazing and with supplementation similar to that in the present study, which indicates that the adopted methodology was adequate to determine the intake of cows. The total dry matter intake in the present study was slightly lower than that cited by the National Research Council (2001) for mid-lactating cows, probably because those recommendations were developed for Holstein cows.

The results showed that Zebu cows kept on pasture and supplemented with concentrate according to MY showed similar feed efficiency among the integrated systems in the Cerrado region, probably due to the similarity in MY and small change in TDMI. These results reject the hypothesis that better herbage nutritional value in the CLFI improves MY and feed efficiency of dairy cows. The feed efficiency observed in the integrated systems (1.35 kg 4% FCM/kg DM) in the present study can be considered average compared to studies that evaluated the FE of dairy cows (Britt et al., 2003; Arndt et al., 2015; Hurley et al., 2018).

#### 4.3. Methane emission

The lower CH<sub>4</sub> production in the rainy season was probably because the animals were consuming a better-quality diet. In addition to the better herbage nutritional value in the rainy season, the amount of concentrate offered per cow was also higher in the rainy than in the dry season. Digestion of herbage cell wall carbohydrates produces mainly acetate and two molecules of H<sup>+2</sup>, which is a precursor of CH<sub>4</sub> production by methanogenic bacteria in the rumen (Sejian et al., 2012). On the other hand, digestion of carbohydrates from concentrated feed mainly produces butyrate (a reaction that produces less H<sup>+2</sup>) and propionate (a reaction that consumes H<sup>+2</sup>) (Moss et al., 2000; Knapp et al., 2014). Due to the intake of a diet with a higher concentrate proportion in the rainy season, there was probably a lower production of H<sup>+2</sup>, which explains the lower emission of enteric CH<sub>4</sub>.

Furthermore, according to Martin et al. (2010), the intake of younger forages with better nutritional value reduces  $CH_4$  emissions due to the higher concentrations of soluble sugars and linolenic acid. Polyunsaturated fatty acids are toxic to gram-positive bacteria, such as *Fibrobacter succinogenes* and *Ruminococcus albus*, through cell wall disruption (Maia et al., 2007). This mechanism may have helped to reduce  $CH_4$  emissions in the rainy season in the present study, since tropical grasses have less senescent material in the rainy season.

Although the herbage nutritional value in the CLFI was better than in the CLI, cows'  $CH_4$  emissions were similar in both systems, which rejects the hypothesis that the better nutritional value in CLFIs reduces enteric  $CH_4$  emissions. This result was not expected, because several studies have shown a reduction in  $CH_4$  emissions due to improvements in diet nutritional value (Martin et al., 2010; Shibata et al., 2010; Beauchemin et al., 2011). According to Seijan et al. (2012), excessive breakdown of nitrogen compounds in the rumen by microorganisms such as *Ruminococcus* spp. and *Butyrivibrio* spp. increase the availability of free carbon skeletons in the rumen, which may have increased CH<sub>4</sub> emissions in the CLFI and generated similar emissions between systems. These compounds can be fermented and increase the production of  $H^{+2}$ , which is a precursor to CH<sub>4</sub>. This hypothesis is supported in the present study by the higher CP and lower NDIP content in the CLFI, which indicates greater availability and digestibility of nitrogen compounds in the rumen.

The average enteric CH<sub>4</sub> emissions for cattle range from 95.9 to 151 g/animal.day (Sejian et al., 2011; Sejian et al., 2012). However, dairy cows have greater emissions due to more intense rumen metabolism. Emissions above these parameters in dairy cows grazing tropical grasses were observed by Primavesi et al. (2004) (331 g/cow.day), Pedreira et al. (2009) (196 g/cow.day), Alves et al. (2017) (491 g/cow.day), Silva et al. (2017) (260 g/cow.day), Congio et al. (2018) (394 g/cow. day) and Jiménez et al. (2021) (383 g/cow.day). Therefore, the average emission of 442 g/cow.day observed in the present study is within the range observed in dairy cows grazing tropical grasses.

The energy loss as CH<sub>4</sub> indicated by the IPCC for grazing dairy cattle is 6.5% ( $\pm$  1%) (Dong et al., 2006), values that are much lower than those found in the present study. However, the authors emphasized that these parameters need to be improved, especially for animals fed on tropical pastures. According to Kurihara et al. (1999), these emission parameters were established mainly with animals fed temperate forages (Johnson and Ward, 1996). Therefore, due to the lower digestibility, higher fiber content and lower soluble carbohydrate content of tropical forages (Archimède et al., 2011), the emissions factors of animals consuming tropical forages may be higher than those cited by the IPCC.

Values close to those established by the IPCC were observed by Hynes et al. (2016) (Ym = 5.6%), Dall-Orsoletta et al. (2019) (Ym = 7.8%) and Moate et al. (2020) (Ym = 6.07%) with dairy cows on temperate grass pasture, mainly ryegrass (*Lolium multiflorum*). On the other hand, higher emissions factors, as observed in the present study, were observed by Primavesi et al. (2004) (Ym = 10.6%) in Girolando cows grazing on tropical grass. The gross energy loss as CH<sub>4</sub> data for dairy cows grazing on tropical grasses are still scarce, which indicates the need for further studies to determine an emission factor more suitable for this situation.

#### 5. Conclusion

The improvement in the herbage nutritional value in CLFI increased intake only in the rainy season and did not change enteric  $CH_4$  emissions, milk yield or feed efficiency of Holstein-Zebu cows in integrated systems in the Brazilian Cerrado region.

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#### CRediT authorship contribution statement

**Roberto Guimarães Júnior:** Investigation, Conceptualization, Data curation, Methodology, Formal analysis, Visualization, Writing – review & editing. **Alan Figueiredo de Oliveira:** Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Isabel Cristina Ferreira:** Investigation, Conceptualization, Data curation, Methodology, Writing – review & editing. **Luiz Gustavo Ribeiro Pereira:** Methodology, Visualization, Writing – review & editing. **Thierry Ribeiro Tomich:** Methodology, Visualization, Writing – review & editing. **Guilherme Lobato Menezes:** Visualization, Data curation, Writing – review & editing. **Lourival Vilela:** Conceptualization, Writing – review & editing. **Ângela Maria** 

**Quintão Lana:** Formal analysis, Visualization, Writing – review & editing.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Supplementary materials

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## 1 CAPÍTULO 3: ARTIGO - PASTURE TRAITS AND CATTLE PERFORMANCE

## 2 IN SILVOPASTORAL SYSTEMS WITH EUCALYPTUS AND UROCHLOA:

## **3 SYSTEMATIC REVIEW AND META-ANALYSIS**

## 4 Artigo publicado na Livestock Science (Impact Factor 1.929)

IUCR SCIENCE 202 (2022) 104975 Contents lists available at ScienceDirect Livestock Science journal homepage: www.elsevier.com/locate/livsci ELSEVIER Pasture traits and cattle performance in silvopastoral systems with Eucalyptus and Urochloa: Systematic review and meta-analysis Alan Figueiredo de Oliveira \*, Guilherme Lobato Menezes \*, Lúcio Carlos Gonçalves \*, Vânia Eloisa de Araújo<sup>b</sup>, Matheus Anchieta Ramirez<sup>a</sup>, Roberto Guimarães Júnior<sup>c</sup>, Diogo Gonzaga Jayme<sup>a</sup>, Ângela Maria Quintão Lana<sup>3</sup> <sup>A</sup>ntimal Science department, Federal University of Minus Geruis, 31270-901, Belo Horizonte, MG, Brazil <sup>1</sup>Denistry department, Pontifical Catholic University of Minus Geruis, 30535-901, Belo Horizonte, MG, Br <sup>2</sup>Brazilian Agricultural Research Corporation – Embrupa Cerrados, 73310-970, Planaltina, DF, Brazil HIGHLIGHTS Forage mass was higher in SPSs with up to 99 trees/ha compared to pasture monoculture. · Forage mass was less reduced in SPSs more than 28m spaced and east-west orientation. No significant improvement was observed in the forage nutritive value in SPSs Total weight gain was higher in SPSs more than 28m spaced and with up to 99 trees/ha. · Total weight gain was reduced with north-south, but not in east-west row orientation. ARTICLE INFO ABSTRACT Keywords: Agroforestry Brachiaria This study evaluated pasture traits and cattle performance in silvopastoral systems (SPSs) with Eucolyptus spp. and Urochlog spp. through a systematic review and meta-analysis. Systematic searches of databases, scientific journals and references of selected articles found 2,639 articles, of which 29 (120 comparisons) were selected. Brazil Comparisons were classified according to the covariates of distance between tree rows, number of trees/ha, tree Integrated syste Trees planting orientation, system age and forage type. Data were submitted to meta-regression followed by subgroup analysis for covariates with a significant effect (P < 0.05) on the response. Data were analyzed in random effects Tropical grass models using mean difference and 95% confidence interval (P < 0.05). Forage mass (FM) was greater for SPSs with up to 99 trees/ha and lower for the other groups, compared to that for grass monoculture. Forage accumulation (FA) was also greater for SPSs with up to 99 trees/ha, but lower for SPSs with more than 300 trees/ha, compared to that for grass monoculture. FM was lower for SPSs of all spacings between tree rows and planting orientations, compared to that for grass monoculture, with the lowest being with smaller spacing and with northsouth planting orientation. FA was lower for SPSs with up to 28m between tree rows, compared to that for grass monoculture, while that for SPSs with more than 28m did not differ. Neutral detergent fiber concentration was lower and crude protein greater for SPSs compared to grass monoculture, while lignin was greater and in vitro dry matter digestibility did not differ, which indicated no significant improvement in nutritive value in SPSs. Average daily gain was greater in SPSs with up to 99 trees/ha, and lower in those with more than 400 trees/ha, than in grass monoculture, with other subgroups not differing. Total weight gain per area (GHA) was lower in SPSs with less than 28m between tree rows or with more than 199 trees/ha, but greater in SPSs with more than 28m or with up to 99 trees/ha, compared to grass monoculture. GHA was lower in SPSs with a north-south orientation compared to grass monoculture, but those with an east-west orientation did not differ. The use of U. brisantha cv. Marandu and an east-west planting orientation are efficient strategies in maintaining FM, FA and GHA. GHA was greater in SPSs with more than 28m between tree rows and with up to 99 trees/ha, than in grass monoculture, which may facilitate the implementation of these SPSs in commercial farms.

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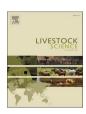
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# Pasture traits and cattle performance in silvopastoral systems with *Eucalyptus* and *Urochloa*: Systematic review and meta-analysis

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#### HIGHLIGHTS

• Forage mass was higher in SPSs with up to 99 trees/ha compared to pasture monoculture.

- Forage mass was less reduced in SPSs more than 28m spaced and east-west orientation.
- No significant improvement was observed in the forage nutritive value in SPSs.
- Total weight gain was higher in SPSs more than 28m spaced and with up to 99 trees/ha.

• Total weight gain was reduced with north-south, but not in east-west row orientation.

#### ARTICLE INFO

Keywords: Agroforestry Brachiaria Brazil Integrated systems Trees Tropical grass

#### ABSTRACT

This study evaluated pasture traits and cattle performance in silvopastoral systems (SPSs) with Eucalyptus spp. and Urochloa spp. through a systematic review and meta-analysis. Systematic searches of databases, scientific journals and references of selected articles found 2,639 articles, of which 29 (120 comparisons) were selected. Comparisons were classified according to the covariates of distance between tree rows, number of trees/ha, tree planting orientation, system age and forage type. Data were submitted to meta-regression followed by subgroup analysis for covariates with a significant effect (P < 0.05) on the response. Data were analyzed in random effects models using mean difference and 95% confidence interval (P < 0.05). Forage mass (FM) was greater for SPSs with up to 99 trees/ha and lower for the other groups, compared to that for grass monoculture. Forage accumulation (FA) was also greater for SPSs with up to 99 trees/ha, but lower for SPSs with more than 300 trees/ha, compared to that for grass monoculture. FM was lower for SPSs of all spacings between tree rows and planting orientations, compared to that for grass monoculture, with the lowest being with smaller spacing and with northsouth planting orientation. FA was lower for SPSs with up to 28m between tree rows, compared to that for grass monoculture, while that for SPSs with more than 28m did not differ. Neutral detergent fiber concentration was lower and crude protein greater for SPSs compared to grass monoculture, while lignin was greater and in vitro dry matter digestibility did not differ, which indicated no significant improvement in nutritive value in SPSs. Average daily gain was greater in SPSs with up to 99 trees/ha, and lower in those with more than 400 trees/ha, than in grass monoculture, with other subgroups not differing. Total weight gain per area (GHA) was lower in SPSs with less than 28m between tree rows or with more than 199 trees/ha, but greater in SPSs with more than 28m or with up to 99 trees/ha, compared to grass monoculture. GHA was lower in SPSs with a north-south orientation compared to grass monoculture, but those with an east-west orientation did not differ. The use of U. brizantha cv. Marandu and an east-west planting orientation are efficient strategies in maintaining FM, FA and GHA. GHA was greater in SPSs with more than 28m between tree rows and with up to 99 trees/ha, than in grass monoculture, which may facilitate the implementation of these SPSs in commercial farms.

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#### 1. Introduction

Silvopastoral systems (SPSs) have been used as a strategy to increase production and reduce environmental impacts of the agricultural sector of many countries (Lemaire et al., 2013). The interaction between plant and animal species produces synergistic effects on total production per area. Improved pasture nutritional value (Paciullo et al., 2014; Santos et al., 2018) and animal thermal comfort (Giro et al., 2019; Oliveira et al., 2019) are examples of the benefits that SPSs can provide that can improve productivity. Therefore, SPSs have the potential to be an important strategy for the improvement of livestock performance and environmental indicators through sustainable intensification.

In Brazil, SPSs predominantly comprise arrangements between *Eucalyptus* spp. and *Urochloa* spp. This is mainly due to the high growth rate and wood quality of *Eucalyptus* spp. trees, the limited number of available other tree species and the high forage mass and quality of *Urochloa* spp. (Balbino et al., 2011; Stape et al., 2010). Some factors, such as competition for water and nutrients and allelopathy, can interfere with system production. Shading is a particularly important factor due to its potential for positive or negative effects on pasture and animal production (Paciullo et al., 2011; Gómez et al., 2012; Santos et al., 2018).

Shading is mainly determined by the spacing and orientation of tree rows and, tree populations and tree row orientation, with greater shading in systems with large tree populations implemented in a northsouth orientation (Paciullo et al., 2009; Santos et al., 2016; Santos et al., 2018). Studies carried out with large tree populations showed great reductions in pasture and animal production (Oliveira et al., 2014; Santos et al., 2016; Santos et al., 2018) with greater than 50% light restriction. On the other hand, studies using arrangements with smaller tree populations showed that SPSs may have similar or superior forage mass compared to grass monoculture (Magalhães et al., 2018; Carvalho et al., 2019; Domiciano et al., 2020). Nonetheless, the assessments of such studies are often based on just a single experiment in one environment, which highlights the need to synthesize these data to better support the implementation of such systems in commercial farms.

The lack of results and clear recommendations regarding the use of SPSs with *Eucalyptus* spp. and *Urochloa* spp. is still an obstacle to largescale use of this strategy in commercial farms (Gil et al., 2015). Furthermore, there has been no systematic review and meta-analysis addressing the effects of different factors present in SPSs on the performance of *Urochloa* spp. and cattle. Greater clarification regarding the effects of tree row spacing and orientation and tree population, as well as system age and forage species, on system responses can provide relevant information regarding the management of SPSs and potentially increase their use. Therefore, this study aimed to evaluate pasture traits and cattle performance in SPSs formed exclusively of *Eucalyptus* spp. and *Urochloa* spp. through a systematic review and meta-analysis.

#### 2. Material and methods

#### 2.1. Protocol and registration

The protocol of the systematic review was submitted and approved by the Open Science Framework with the title: Effect of shading on the tropical pastures and cattle performances in silvopastoral systems: Systematic review and meta-analysis (https://doi.org/10.17605/OSF.IO/ 7R2PH). The study followed the recommendations proposed by the guideline Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Page et al., 2021).

#### 2.2. Eligibility criteria

Replicated and randomized studies published in Portuguese, English or Spanish in scientific journals that evaluated the performance of SPSs with *Eucalyptus* spp. and *Urochloa* spp. were included. Criteria for inclusion followed the PICO template (Thomas et al., 2019): **Population**: *Urochloa* spp. pasture; **Intervention**: *Eucalyptus* spp. shading; **Comparison**: *Urochloa* spp. in grass monoculture; **Primary outcomes (animal performance)**: average daily gain (kg/animal/day), total weight gain per area (kg/ha; GHA), stocking rate [animal unit (AU)/ha; SR] and milk production (kg/cow/day) and **Secondary outcomes (pasture traits)**: crude protein (% DM; CP), neutral detergent fiber (% DM), acid detergent fiber (% DM), lignin (% DM), *in vitro* dry matter digestibility (% DM; IVDMD), forage mass (Mg/ha; FM), forage accumulation (Mg/ha; FA), forage density (kg DM/cm/ha), pasture height (cm), tiller number (tiller/m<sup>2</sup>), leaf area index, specific leaf area (cm<sup>2</sup>/g), percentage of plant leaf (%), percentage of plant stem (%) and percentage of plant senescent material (%; PPSM).

#### 2.3. Information source and data search

The search strategy for articles was systematically carried out in Scopus, Embase, Web of Science and MEDLINE-Pubmed databases and directly in scientific journals and the references of articles selected by the systematic review. The terms used to characterize the POPULATION were "tropical pasture", "tropical forage", "tropical grassland", "full sun pasture", "pasture", "grassland", "*Brachiaria*" and "*Urochloa*". The terms used to characterize the INTERVENTION were "silvopastoral systems", "livestock-forest integration", "integrated livestock-forest system", "integrated systems", "shaded pasture", "shade", "light restriction" and "eucalyptus". The last survey was carried out on 18 August 2021.

#### 2.4. Selection of studies and data collection process

The studies were consolidated into a database after removing all duplicates using EndNote software. Studies were extracted and peer reviewed using Excel. The following exclusion criteria were used: **Population type**: studies conducted without *Urochloa* spp.; **Intervention type**: studies that did not use *Eucalyptus* spp. shading; **Study type**: case studies, descriptive studies, non-primary studies, patents, studies without a grass monoculture control and studies without estimates of the mean and variation for the evaluated variables. Divergences between researchers were solved with the help of a third researcher.

A total of 2,639 studies were selected from the surveyed databases and, after excluding duplicates, the titles and abstracts of 2,470 articles were extracted (Fig. 1). After reading the titles and abstracts, 129 articles were selected for reading of the full text, with population type being the most frequent cause of exclusion at this phase (1,702 articles). After reading the full text, 29 studies were selected, with intervention type being the most frequent cause of exclusion at this phase (92 studies). A total of two articles were included by manual search. Data were extracted after study evaluation.

#### 2.5. Statistical analysis

Data analysis was performed using the "meta" package of R software (R Core Team, 2019). Data were classified according to the covariates of distance between tree rows (*Eucalyptus* spp. with 1 to 14m between tree rows, *Eucalyptus* spp. with 15 to 28m between tree rows or *Eucalyptus* spp. with more than 28m between tree rows), tree population (up to 99 trees/ha, between 100 to199 trees/ha, between 200 to 299 trees/ha, between 300 to 399 trees/ha, between 400 to 499 trees/ha or more than 499 trees/ha), tree row orientation (east-west or north-south), system age since tree planting (up to three years, between four and five years, between six and seven years or more than seven years) and forage species [*Urochloa brizantha* (syn. *Brachiaria brizantha* - Hochst. ex A. Rich. - Stapf. R. D. Webster) cv. Marandu and cv. Piatã or *Urochloa decumbens* (syn. *Brachiaria decumbens* Stapf R. D. Webster)]. This classification was made according to the characteristics of the selected studies and the authors' experience in these systems.

Mean difference (MD) was used as a dependent variable in the mixed

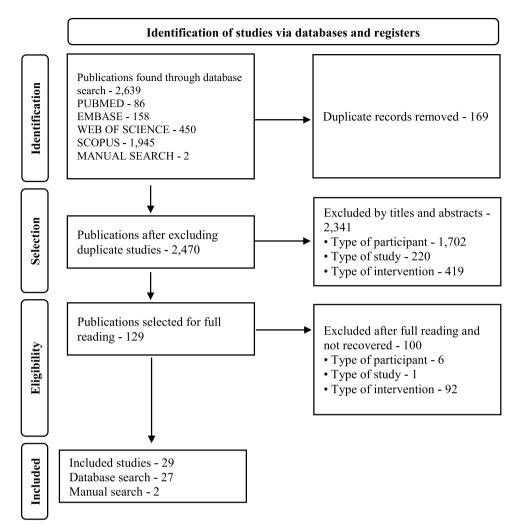


Fig. 1. Flowchart of the selection process of studies included in the meta-analysis

meta-regression models (Viechtbauer, 2010) to identify the effect of each covariate on pasture and animal variables according to the model:

$$\theta_i = \beta_0 + \beta_d x_{id} + \beta_t x_{it} + \beta_o x_{io} + \beta_a x_{ia} + \beta_s x_{is} + u$$

Where:  $\theta_i$  refers to the true overall effect,  $\beta_0$  refers to the overall mean,  $\beta_d x_{id}$  refers to the effect of the covariate of distance between tree rows for the i-th study,  $\beta_c x_{it}$  refers to the effect of the covariate of tree population for the i-th study,  $\beta_o x_{io}$  refers to the effect of the covariate of tree row orientation for the i-th study,  $\beta_a x_{ia}$  refers to the effect of the covariate of system age for the i-th study,  $\beta_s x_{is}$  refers to the effect of the covariate of forage species for the i-th study, and  $u_i \sim N(0; \frac{2}{7})$  with  $\frac{2}{7}$  referring to the amount of residual heterogeneity among the true effects.

Wald's multiparametric test was used to test the effects of covariates on responses (Viechtbauer, 2010). The adjusted  $R^2$  value was calculated by eq (1):

$$R^{2}(\%) = (\sigma_{o}^{2} - \sigma^{2}) / \sigma_{o}^{2}$$

Where:  $R^2$  refers to variation between studies,  $\sigma_o^2$  refers to variation between studies without the covariate in the models, and  $\sigma^2$  refers to variation between studies with the covariate in the models. When a covariate had a significant effect on the response, subgroup analysis was performed.

For subgroup analysis, data were analyzed using random effects models using MD with 95% confidence interval (P < 0.05) according to the model:

$$Y_i = \mu + \xi i + \varepsilon i$$

Where:  $\xi i$  refers to the difference between the grand mean ( $\mu$ ) and the true mean ( $\theta_i$ ) for study i ( $\xi i = \theta_i - \mu$ ), and  $\epsilon i$  refers to the difference between the true mean for study i ( $\theta_i$ ) and the observed mean ( $Y_i$ ) for study i ( $\epsilon i = Y_i - \theta_i$ ) (Borenstein et al., 2010).

The mean difference was calculated according to eq (2):

$$MD = M_{1i} - M_{2i}$$

Where:  $M_{1i}$  and  $M_{2i}$  refer to the mean value of treatment and grass monoculture, respectively. When significant, positive values favor SPSs and negative values favor grass monoculture (Deeks et al., 2019).

The studies were weighted by the inverse of the variance. Heterogeneity ( $I^2$ ) was evaluated based on the criteria:  $I^2$  up to 30%,  $I^2$  between 31 and 75% and  $I^2$  greater than 75% indicating low, moderate and high heterogeneity (P < 0.1), respectively (Deeks et al., 2019).  $I^2$  means that the confidence intervals for the results of individual studies have limited overlap, which generally indicates the presence of statistical heterogeneity.  $I^2$  describes the percentage of variability in effect estimates that is due to heterogeneity rather than sampling error (chance). Heterogeneity was calculated using eq (3):

$$I^2 = [(Q - df) / Q] \times 100\%$$

Where: Q refers to the  $\text{Chi}^2$  statistic and df refers to degrees of freedom (Higgins and Thompson 2002, Higgins et al., 2003).

Publication bias risk was assessed by testing the symmetry between the standard deviation (SD – accuracy parameter) and MD (true effect parameter) using funnel plot (Higgins et al., 2019) and by the Egger's regression method between MD and SD (Egger et al., 1997).

#### 3. Results

#### 3.1. Study characteristics

Only studies implemented in Brazil with the evaluation of SPSs with *Eucalyptus* spp. and *Urochloa* spp. were used. SPSs with *Eucalyptus* spp. with 1 to 14m between tree rows were used in 27.5% of the comparisons, with 15 to 28m between tree rows in 37.5% of the comparisons and with more than 28m between tree rows in 35.0% of the comparisons (Table 1). SPSs with up to 99 trees/ha were used in five comparisons, with 100 to 199 trees/ha in 22 comparisons, with 200 to 299 trees/ha in 34 comparisons, with 300 to 399 trees/ha in 33 comparisons, with 400 to 499 trees/ha in 11 comparisons and with more than 499 trees/ha in 15 comparisons. SPSs with tree rows with east-west orientation were used in 101 comparisons and with north-south orientation in 19.

The forage species used were U. brizantha cv. Piatã (50.0% of

comparisons), *U. brizantha* cv. Marandu (28.3% of comparisons) and *U. decumbens* (21.7% of comparisons) (Table 1). Five comparisons were made in systems of up to three years of age, 76 of four or five years of age, 18 of six or seven years of age and 21 of more than seven years of age since tree planting. Twenty-three studies lasted for up to one year, four lasted between one to two years and two lasted longer than two years. No study evaluated the performance of dairy cows in SPSs exclusively with *Eucalyptus* spp. and *Urochloa* spp. Of the 29 studies, 18 evaluated animal performance, with the Nellore breed and its crosses with other zebuine and taurine breeds being the most used.

Funnel plot evaluation of publication bias risk showed a uniform distribution of the studies and low bias risk (Fig. 2). However, Egger's regression showed the variables of FM, leaf area index, PPSM, tiller number and CP to have P < 0.05 and all other variables to have P > 0.05 (Table 2).

#### 3.2. Agronomic traits

The general meta-analysis showed that FM, FA, forage density, PPSM and tiller number were lower, and specific leaf area, percentage of plant leaf and percentage of plant stem were greater, for SPSs compared to

#### Table 1

Productive characteristics of the silvopastoral systems evaluated in the selected studies

Author (Year)*	Country	Distance between tree rows <sup>@</sup>	Tree population @	Forage species	Experiment duration	System age since tree planting	Tree row orientation
Aranha et al. (2019)	Brazil	Eu15-28	196/448	<i>U. brizantha</i> cv. Marandu	6 months	5 years	East-west
Araujo et al. (2013)	Brazil	Eu1-14	250/400/1650	U. decumbens	1 year	2 years	East-west
Barros et al. (2018)	Brazil	Eu1-14/Eu15-28	227/357	U. brizantha cv. Piatã	7 months	6 years	East-west
Bonini et al. (2016)	Brazil	Eu15-28	200/500	<i>U. brizantha</i> cv. Marandu	1 month	2 years	East-west
Carvalho et al. (2019)	Brazil	More28m	90/135	<i>U. brizantha</i> cv. Marandu	1 year	5 years	East-west
Crestani et al. (2017)	Brazil	More28m	338/714	U. brizantha cv. Piatã	1 year	4 years	East-west
Domiciano et al. (2016)	Brazil	More28m	270	<i>U. brizantha</i> cv. Marandu	1 year	4 years	East-west
Domiciano et al. (2020)	Brazil	More28m	90/270	<i>U. brizantha</i> cv. Marandu	1 year	5 years	East-west
Geremia et al. (2018)	Brazil	Eu15-28/More28m	338/714	U. brizantha cv. Piatã	1 year	4 years	East-west
Gomes et al. (2020A)	Brazil	More28m	135	<i>U. brizantha</i> cv. Marandu	2 years	6 years	East-west
Gomes et al. (2020 <b>B)</b>	Brazil	More28m	135	<i>U. brizantha</i> cv. Marandu	2 years	6 years	East-west
ana et al. (2016).	Brazil	More28m	150	<i>U. brizantha</i> cv. Marandu	7 months	15 years	East-west
.ima et al. (2018)	Brazil	More28m	165	U. decumbens	2 years	18 years	North-south
lopes et al. (2017A)	Brazil	More28m	165	U. decumbens	6 months	13 years	North-south
opes et al. (2017B).	Brazil	More28m	165	U. decumbens	6 months	13 years	North-south
Magalhães et al. (2018)	Brazil	More28m	90/270	<i>U. brizantha</i> cv. Marandu	1 year	5 years	East-west
Martins et al. (2020)	Brazil	Eu15-28	227	U. brizantha cv. Piatã	1 year	6 years	East-west
Vascimento et al. (2019)	Brazil	More28m	270	<i>U. brizantha</i> cv. Marandu	1 year	4 years	East-west
Nascimento et al. (2021)	Brazil	More28m	270	<i>U. brizantha</i> cv. Marandu	1 year	4 years	East-west
Neves et al. (2021)	Brazil	Eu15-28	187/446	<i>U. brizantha</i> cv. Marandu	3 years	4 years	East-west
Oliveira et al. (2013)	Brazil	Eu1-14/Eu15-28	227/357	U. brizantha cv. Piatã	1 year	3 years	East-west
Pereira et al. (2015)	Brazil	Eu1-14	250/400/1650	U. decumbens	1.5 year	5 years	East-west
Pereira et al. (2021)	Brazil	Eu1-14/Eu15-28	227/357	U. brizantha cv. Piatã	1 year	8 years	East-west
Pezzopane et al. (2020)	Brazil	Eu15-28	333	U. brizantha cv. Piatã	2 years	4 years	East-west
Rodrigues et al. (2018)	Brazil	Eu1-14	250	<i>U. brizantha</i> cv. Marandu	3 months	8 years	East-west
Santos et al. (2016)	Brazil	Eu1-14/Eu15-28	417/715	U. brizantha cv. Piatã	1 year	4 years	North-south
Santos et al. (2018)	Brazil	Eu1-14/Eu15-28	417/715	U. brizantha cv. Piatã	1 year	4 years	North-south
Silva et al. (2020)	Brazil	More28m	270	U. brizantha cv. Marandu	1 year	7 years	East-west
Xavier et al. (2014)	Brazil	More28m	165	U. decumbens	1 year	5 years	North-south

\* The papers are listed in supplementary file 1

 $^{(0)}$ , The bars indicate that more than one group was present in the study; Eu1-14 = Eucalyptus with 1 to 14m between tree rows, Eu15-28 = Eucalyptus with 15 to 28m between tree rows, More28m = Eucalyptus with more than 28m between tree rows.

grass monoculture (P < 0.05) while pasture height and leaf area index did not differ (P > 0.05) (Table 2). The meta-regression showed that FM and FA were altered by all covariates (Table 3) and they accounted for 45.4 and 54.3% of the data variation, respectively. FM was lower for all SPSs with more than 99 trees/ha compared to grass monoculture, with the lowest being for SPSs with larger tree populations (P < 0.05) (Fig. 3). However, FM was greater for SPSs with up to 99 trees/ha compared to grass monoculture. FA was greater for SPSs with up to 99 trees/ha compared to grass monoculture and less for SPSs with more than 300 trees/ha, while SPSs with tree populations between 100 to 299 trees/ha did not differ (Table 4). Considering the spacing between tree rows, FM was lower than grass monoculture for all SPSs, being lowest with smaller spacing (P < 0.001). FA was lower for SPSs with up to 28m between tree rows compared to grass monoculture, but SPSs with more than 28m did not differ. All tree row orientations had lower FM and FA (P < 0.001) compared to grass monoculture, the lowest being with a north-south orientation. FM was lower than grass monoculture (P < 0.05) for SPSs of all ages and all forage grasses, the lowest being with *U. brizantha* cv. Marandu. FA was lower for SPSs with *U. brizantha* cv. Piatâ and *U. decumbens* compared to grass monoculture, but did not differ for *U. brizantha* cv. Marandu.

Forage density was lower for SPSs of all tree row spacings, tree population, tree row orientations and forage species (P < 0.05), being lowest with lower tree row spacing, greater tree population, tree rows with north-south orientation and with *U. brizantha* cv. Piatã (Tables 3 and 4). Heterogeneity was high ( $I^2 > 83.7\%$ ; P < 0.1) for the subgroups with *Eucalyptus* spp. with more than 28m between tree rows, with 100 to 199 trees/ha, with both tree row orientations and with all forage species. Pasture height differed with spacing between tree rows and forage species (Table 3). Pasture height was lower for SPSs with 1 to 14m

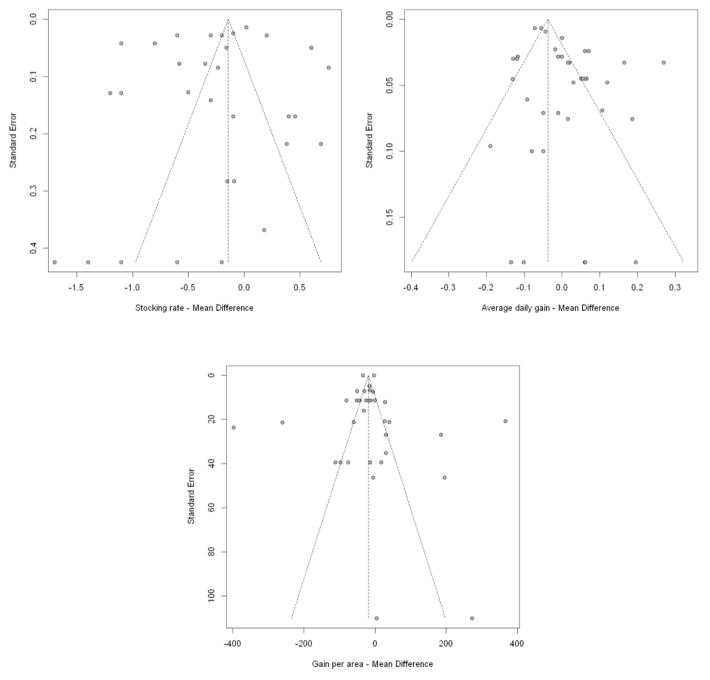


Fig. 2. Funnel plot for stocking rate, average daily gain and total weight gain per area in beef cattle in silvopastoral system with Eucalyptus spp. and Urochloa spp.

#### Table 2

General meta-analysis of effect of Eucalyptus spp. shade in silvopastoral system on Urochloa spp. and animal performances

Variable	N <sup>@</sup>	MD (CI 95%) <sup>#</sup>		Heterogeneity <sup>\$</sup>	
		Random effect	P-value	I <sup>2</sup> (%)	P-value
Agronomic variables					
Forage mass (Mg/ha)	108	-1.32 [-1.46; -1.18]	< 0.001	95.2	< 0.001
Forage accumulation (Mg/ha)	52	-1.09 [-1.30; -0.879]	< 0.001	97.3	< 0.001
Forage density (kg DM/ha/cm)	25	-24.8 [-30.8; -18.7]	< 0.001	94.4	< 0.001
Pasture height (cm)	47	-1.99 [-4.25; 0.275]	0.085	100	< 0.001
Leaf area index	19	-0.136 [-0.456; 0.184]	0.406	96.0	< 0.001
Specific leaf area (cm <sup>2</sup> /g)	10	18.3 [9.91; 26.6]	< 0.001	96.2	< 0.001
Leaf percentage (%)	34	2.15 [0.644; 3.65]	0.005	94.6	< 0.001
Stem percentage (%)	34	1.46 [0.464; 2.45]	0.004	88.0	< 0.001
Dead material percentage (%)	26	-2.71 [-3.78; -1.64]	< 0.001	94.5	< 0.001
Tiller number (tiller/m <sup>2</sup> )	15	-238 [-335; -142]	< 0.001	91.4	< 0.001
Nutritional variables					
Crude protein (% DM)	59	1.83 [1.38; 2.28]	< 0.001	99.2	< 0.001
Neutral detergent fiber (% DM)	28	-1.58 [-2.45; -0.708]	< 0.001	98.2	< 0.001
Acid detergent fiber (% DM)	16	-0.419 [-1.16; 0.326]	0.269	99.0	< 0.001
Lignin (% DM)	3	1.11 [0.521; 1.70]	< 0.001	23.4	0.271
In vitro dry matter digestibility (% DM)	12	-0.385 [-1.89; 1.12]	0.616	69.0	< 0.001
Animal performance variables					
Stocking rate (UA/ha)	34	-0.234 [-0.372; -0.097]	< 0.001	98.4	< 0.001
Average daily gain (kg/animal/day)	37	0.005 [-0.021; 0.031]	0.687	87.8	< 0.001
Gain per area (kg/ha)	35	-20.6 [-30.2; -11.1]	< 0.001	99.9	< 0.001

 $^{@}$  N = number of comparisons between silvopastoral system and grass monoculture.  $^{\#}$  MD = mean differences between silvopastoral system and grass monoculture treatments.  $^{\$}$  I<sup>2</sup> = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for  $\chi 2$  (Q) test of heterogeneity.

between tree rows (MD = -7.82; P < 0.001) and greater for SPSs with more than 28m (MD = 1.96; P < 0.001) compared to grass monoculture, but did not differ with 15 to 28m (P = 0.06) (Table 4). Height of *U. decumbens* was lower for SPSs than for grass monoculture (MD = -10.5; P < 0.001), but did not differ for other forage species. Heterogeneity was high (I<sup>2</sup> > 84.9%; P < 0.1) for subgroups with 1 to 28m between tree rows and with all forage species.

Leaf area index was affected by all covariates and they were responsible for 97.1% of the variation in the data (Table 3). Leaf area index was lower for SPSs with up to 28m between tree rows than for grass monoculture (P < 0.001), but did not differ with more than 28m between tree rows (P = 0.140) (Table 4). Leaf area index was greater for SPSs with 100 to 199 trees/ha (P < 0.001) and lower for SPSs with more than 299 trees (P < 0.05), compared to monoculture. Leaf area index was lower for SPSs with tree rows with north-south orientation (MD = -0.659; P < 0.001) compared to grass monoculture, but did not differ for east-west orientation (P = 0.86). Leaf area index was greater for SPSs with U. brizantha cv. Marandu (MD = 0.414; P < 0.001), but lower for SPSs with U. brizantha cv. Piatã (MD = -0.707; P < 0.001), compared to grass monoculture. Heterogeneity was high ( $I^2 > 70.8$ ; P < 0.1) for subgroups with more than 14m between tree rows, with all tree row orientations and with all forage species, and for SPSs of four to five years of age since tree planting. Specific leaf area differed with spacing between tree rows and tree population and the covariates explained 19% of the variation in the data (Table 3). Specific leaf area was greater for SPSs with 1 to 14m (MD = 39.3; P = 0.05) and with more than 28m (MD = 11.3; P < 0.001) compared to grass monoculture (Table 4). Heterogeneity was high for all subgroups ( $I^2 > 77.2$ ; P < 0.1).

PPSM was affected by spacing between tree rows, tree row orientation and forage species (Table 3). PPSM was greater for SPSs with 1 to 14m (P = 0.009), but lower for the other subgroups (P < 0.05), compared to grass monoculture (Table 4). PPSM was lower for SPSs with east-west orientation (MD = -2.31; P < 0.001) compared to grass monoculture, but did not differ for those with north-south orientation (P = 0.13). PPSM was lower for SPSs with *U. brizantha* cv. Marandu and *U. brizantha* cv. Piatã than for grass monoculture, but did not differ for those with *U. decumbens*. Heterogeneity was high (I<sup>2</sup> > 91.8; P < 0.1) for subgroups with more than 15m between tree rows and with all tree row orientations and forage species. Tiller number was affected by tree population and system age and the covariates explained 52.4% of the variation in the data (Table 3). Tiller number was lower for all SPSs (P < 0.05) compared to grass monoculture, being the lowest for SPSs with 100 to 199 trees/ha and six to seven years of age since tree planting (Table 4). Heterogeneity was high (I<sup>2</sup> > 74.7; P < 0.001) for subgroups with 100 to 199 trees/ha and greater than six years of age since tree planting.

#### 3.3. Nutritional value

The general meta-analysis showed that neutral detergent fiber was lower for SPSs than for grass monoculture. CP and lignin were greater for SPSs (P < 0.05) than in grass monoculture, but acid detergent fiber and IVDMD did not differ (P > 0.05) (Table 2). CP was affected by system age and the covariates explained 55.6% of the variation in the data (Table 3). CP was greater for SPSs of all ages (P < 0.05), than for grass monoculture, being greatest in older systems (Table 5). Heterogeneity was high (I<sup>2</sup>>93.5; P < 0.001) for all subgroups. IVDMD was affected by tree row orientation and the covariates explained 3.82% of the variation in the data (Table 3). IVDMD was lower for SPSs with tree rows with north-south orientation (MD = -1.62; P = 0.05) than for grass monoculture, but did not differ for those with east-west orientation (P = 0.29).

#### 3.4. Animal performance

The general meta-analysis showed that SR and GHA were lower in SPSs (P < 0.05) than in grass monoculture, while average daily gain did not differ (P > 0.05) (Table 2). SR was affected by all covariates and they explained 6.11% of the variation in the data (Table 3). SR was lower in SPSs with up to 28m between tree rows (P < 0.001) compared to grass monoculture, but did not differ in those with more than 28m (Table 6). SR was greater in SPSs with up to 99 trees/ha (MD = 0.637; P < 0.001) and lower in SPSs with more than 200 trees/ha (P < 0.05) compared to grass monoculture. SR was lower in SPSs with tree rows with east-west orientation (MD = -0.176; P = 0.02) than in grass monoculture but did not differ in those with north-south orientation (P = 0.06). SR was lower in SPSs with *U. brizantha* cv. Piatã (MD = -0.535; P < 0.001) than in grass monoculture but did not differ in those with *U. brizantha* cv. Marandu. Heterogeneity was high (I<sup>2</sup>>71.8; P < 0.1) for all tree row spacings, tree row orientations, system ages, forage species and with

#### Table 3

Meta-regression of the effect of distance between tree rows, tree population, tree row orientation, system age and forage species on mean differences (MD) between silvopastoral system and grass monoculture for *Urochloa* spp. and animal performance

Dependent variable	Meta-regression	n parameters (P-value) <sup>#</sup>					$\mathbf{N}^{\neg}$	R <sup>2</sup> (%) <sup>¢</sup>	Funnel plot <sup>£</sup>
(Y, MD)@	Intercept	Distance between tree rows	Tree population	Tree row orientation	System age	Forage species		(,	Free
Agronomic variables									
FM (Mg/ha)	4.68 (P <	0.217 (P < 0.001)	0.411 (P <	0.002 (P =	0.019 (P =	0.104 (P <	108	45.4	0.001
	0.001)		0.001)	0.001)	0.005)	0.001)			
FA (Mg/ha)	8.78 (P =	0.094 (P < 0.001)	0.368 (P <	0.066 (P <	0.032 (P <	0.135 (P <	52	54.3	0.054
	0.003)		0.001)	0.001)	0.001)	0.001)			
FD (kg DM/ha/	-45.9 (P <	0.578 (P < 0.001)	0.657 (P <	0.156 (P =	0.084 (P =	0.464 (P <	25	62.2	0.924
cm)	0.001)		0.001)	0.026)	0.311)	0.001)			
PH (cm)	21.7 (P =	-0.147 (P < 0.001)	-0.145 (P =	-0.001 (P =	0.001 (P =	0.001 (P <	47	0	0.942
	0.077)		0.440)	0.669)	0.939)	0.001)			
LAI	-1.36 (P <	0.743 (P < 0.001)	0.977 (P <	0.561 (P =	0.886 (P <	0.891 (P <	19	97.1	0.017
	0.001)		0.001)	0.012)	0.001)	0.001)			
SLA (cm <sup>2</sup> /g)	39.0 (P <	0.190 (P = 0.022) <sup>\$</sup>	0.190 (P =	-0.198 (P =	-0.198 (P =	-0.198 (P =	10	19	0.137
	0.001)		0.022) \$	0.087) *	0.087) *	0.087) *			
LP (%)	2.89 (P =	-0.132 (P = 0.445)	0.020 (P =	0.146 (P =	0.082 (P =	0.299 (P =	34	0	0.082
	0.585)		0.929)	0.181)	0.129)	0.317)			
SP (%)	2.27 (P =	-0.016 (P = 0.367)	-0.295 (P =	-0.023 (P =	-0.063 (P =	-0.059 (P =	34	0	0.418
	0.471)		0.657)	0.937)	0.528)	0.893)			
DMP (%)	-0.458 (P =	-0.706 (P = 0.049)	-0.802 (P =	0.079 (P =	0.019 (P =	0.123 (P =	26	0	0.003
	0.913)		0.285)	0.027)	0.752)	0.049)			
TN (tiller/m <sup>2</sup> )	-720 (P <	-0.060 (P = 0.598)	0.086 (P =	-0.099 (P =	0.560 (P <	-0.099 (P =	15	52.4	0.003
( )	0.001)		0.023)	0.485)	0.001)	0.485)			
Nutritional variables				,	,	,			
CP (% DM)	3.79 (P =	-0.073 (P = 0.228)	0.068 (P =	-0.009 (P =	0.401 (P <	0.248 (P =	59	55.6	0.002
	0.060)	01070 (1 01220)	0.562)	0.097)	0.001)	0.091)	0,5	00.0	0.002
NDF (% DM)	3.59 (P =	0.017 (P = 0.648)	0.015 (P =	-0.023 (P =	-0.023 (P =	-0.022 (P =	28	0	0.646
()) Diii)	0.431)	5.017 (I - 0.0 f0)	0.878)	0.960)	0.832)	0.519)	20	0	0.0 10
ADF (% DM)	7.53 (P =	-0.046 (P = 0.100)	-0.059 (P =	-0.012 (P =	0.004 (P =	0.020 (P =	16	0	0.55
	0.011)	5.0 10 (I = 0.100)	0.148)	0.562)	0.373)	0.020 (1 = 0.004) <sup>©</sup>	10	0	0.00
LIG (% DM)	2.05 (P =	_	-	0.329 (P =	-	0.329 (P =	3	32.9	0.999
	2.03 (P = 0.025)	-	-	0.329 (P = 0.276) ~	-	0.329 (P = 0.276) ~	5	34.9	0.999
IVDMD (% DM)	3.42 (P =	-0.013 (P = 0.207)	-0.359 (P =	$0.270) \approx$ 0.310 (P =	-0.124 (P =	-0.146 (P =	13	3.82	0.854
	0.446)	0.010(1 - 0.207)	-0.339 (F = 0.936)	0.037)	-0.124 (F = 0.816)	-0.140 (F = 0.638)	10	5.02	0.034
Animal performance			0.930)	0.0373	0.010)	0.030)			
Animai performance SR (AU/ha)	-0.179 (P =	0.148 (P < 0.001)	0.340 (P <	-0.123 (P =	-0.388 (P =	-0.126 (P <	34	6.11	0.076
on (AU/IIA)		0.148 (P < 0.001)		-	-		34	0.11	0.076
ADC (leg (anim -1))	0.815)		0.001)	0.015)	0.047)	0.001)	07	0	0.060
ADG (kg/animal/	-0.008 (P = 0.062)	0.205 (P = 0.066)	0.289 (P <	-0.211 (P =	-0.105 (P = 0.500)	-0.289 (P = 0.222)	37	0	0.069
day)	0.962)	0.000 (D < 0.001)	0.001)	0.132)	0.509)	0.232)	25	0.046	0.001
GHA (kg/ha)	-194 (P =	0.002 (P < 0.001)	-0.747 (P <	-0.067 (P <	-0.067 (P = 0.071)	-0.066 (P <	35	0.046	0.091
	0.001)		0.001)	0.001)	0.071)	0.001)			

\*

<sup>(a)</sup> FM = forage mass, FA = forage accumulation, DM = dry matter; FD = forage density, PH = Pasture height, LAI = leaf area index, SLA = specific leaf area, LP = leaf percentage, SP = stem percentage, DMP = dead material percentage, TN = tiller number, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, LIG = lignin, IVDMD = *in vitro* dry matter digestibility, ST = stocking rate, AU = animal unit (450 kg live weigh), ADG = average daily gain, GHA = gain per area. <sup>#</sup> Distance between tree row = Eucalyptus 1 to 14m spaced, Eucalyptus 15 to 28m spaced, Eucalyptus more than 28m spaced; Tree population = Up to 99 trees, Between 100 to 199, Between 200 to 299, Between 300 to 399, Between 400 to 499, More than 499; Tree row orientation = East-west, North-South; System age since tree planting = Up to three years, Between four and five years, Between six and seven years, More than Seven years; Forage species = *Urochloa brizantha* cv. Marandu, *Urochloa brizantha* cv. Piatã, *Urochloa decumbens*. ¬N = number of comparisons between silvopastoral system and grass monoculture. <sup>€</sup> R<sup>2</sup> = proportion of the between-study variance (heterogeneity) explained by the distance between tree rows, tree population, tree row orientation, system age and forage species covariates. <sup>£</sup> Egger's regression asymmetry test. <sup>\$</sup>

<sup>~</sup> These subgroups had the same comparisons, and for this reason had the same regression parameters. <sup>©</sup> In this analysis only Marandu subgroup was different, however how there was only one comparison in this subgroup, it was not considered and the subgroup analysis was not performed.

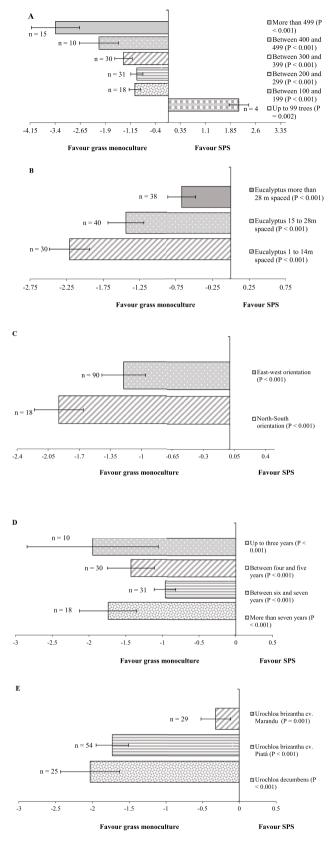
more than 100 trees/ha. Average daily gain was affected by tree population (Table 3). Average daily gain was greater for animals in SPSs with up to 99 trees/ha (MD = 0.148; P < 0.001) than for those in grass monoculture, but was lower for those in SPSs with more than 400 trees/ha (MD = -0.048; P < 0.001).

GHA was affected by tree row spacing, tree population, tree row orientation and forage species and the covariates explained 0.046% of the variation in the data (Table 3). GHA was lower in SPSs with less than 28m between tree rows, but was greater in those with more than 28m, compared to in grass monoculture (P < 0.05) (Fig. 4). GHA was also greater in SPSs with up to 99 trees/ha, but lower in those with more than 200 trees/ha, compared to in grass monoculture. GHA was lower in SPSs with north-south orientation (P = 0.03) than in grass

monoculture, but did not differ in those with east-west orientation. GHA was lower in systems with *U. brizantha* cv. Piatã (P < 0.001) and greater in SPSs with *U. brizantha* cv. Marandu (P = 0.003) compared to in grass monoculture.

#### 4. Discussion

This is the first systematic review and meta-analysis study about pasture and animal performances in SPSs with *Eucalyptus* spp. and *Urochloa* spp. Although knowledge about these systems has evolved significantly in recent years, there remains a need to summarize scientific results in order to better support field recommendations for the adoption of SPSs under different conditions. Therefore, the results of this



**Fig. 3.** Subgroup analysis for forage mass (Mg/ha) according to trees population (A), distance between rows (B), row orientation (C), system age (D) and forage species (E) in silvopastoral system with *Eucalyptus* spp. and *Urochloa* spp. N, indicates the number of comparisons in each subgroup.

study are important for the successful implementation of this system in commercial farms.

#### 4.1. Study characteristics

All studies with SPSs established with Eucalyptus spp. and Urochloa spp. were carried out in Brazil, which shows the important role of this country in the development of this system. Wood valorization on the Brazilian market, rapid initial growth and relative ease of cultivation are factors that drive Eucalyptus spp. use in Brazil (Balbino et al., 2011). Studies with dairy cows in SPSs exclusively with Eucalyptus spp. and Urochloa spp. are scarce. The only studies to have evaluated the performance of these animals in SPSs in Brazil are Paciullo et al. (2014) (established with Eucalyptus sp. + Acacia mangium and Urochloa sp.) and Martins et al. (2020) (established with Eucalyptus sp. and Megathyrsus sp.). However, these studies were not included here because they did not exclusively integrate Eucalyptus spp. and Urochloa spp. The lack of studies with dairy cattle is probably due to the greater cost and complexity of handling animals in experimental stations compared with beef cattle and the difficulty of obtaining an adequate number of animals with similar physiological characteristics to establish robust studies.

Most of the SPSs used 15 to 28m between tree rows, from 200 to 399 trees/ha, tree rows with east-west orientation and *U. brizantha* cv. Piatã. The combination of these factors determines the competition for natural resources among system components, pasture shading level and animal production. Therefore, there is a tendency to use smaller tree populations and more productive forages to maintain animal performance and increase total system production (Pontes et al., 2021). Most studies were of short duration (up to one year), which is explained by the operational complexity of developing long-term experiments. However, it is important that longer evaluations be carried out because tree growth can increase shading and reduce pasture production over years and indicate the need for management such as pruning.

Funnel plot is a subjective analysis that evaluates the accuracy integrated with the true effect of each comparison. Thus, the inclusion of comparisons with low accuracy (broad confidence interval) and similar true effect (only positive or negative effect) indicates a high risk of publication bias and reduces the reliability of results (Higgins et al., 2019). The comparisons of the present study showed a uniform distribution for animal performance variables in the funnel plot (symmetrical distribution with positive and negative values) and high accuracy, which indicates no publication bias.

Egger's regression is a complementary test to funnel plot that assesses the probability of a symmetrical distribution of studies (Higgins et al., 2019). The variables FM, leaf area index, PPSM, tiller number and CP had significant effects on Egger's regression (P < 0.05), which indicates an asymmetric distribution and the need to be cautious in interpreting results involving these variables. This wide dispersion of data was due to some comparisons involving large differences between SPSs and grass monoculture, such as for systems with up to 14m between tree rows. However, all collected values are commonly observed in SPSs with *Eucalyptus* spp. and *Urochloa* spp., and thus data deletion was not performed.

#### 4.2. Pasture agronomic traits

Forage mass and forage accumulation are among the most important agronomic traits of grasses in SPSs, as they indicate pasture productive capacity. The evaluated covariates explained only 45.4% of FM and 54.3% of FA. Other factors, such as seasons, soil characteristics, location within SPSs and distance from the tree row are factors that alter FM and FA (Gomes et al., 2019; Nascimento et al., 2019; Gomes et al., 2020). However, we did not assess these factors because they are difficult to control during system planning and were not adequately described in all studies, which made it impossible to classify comparisons by these covariates. In addition, FM showed an asymmetric distribution in

#### Table 4

Subgroup analysis for forage agronomic variables according to tree population, distance between tree rows, tree row orientation, system age and forage species in silvopastoral system with *Eucalyptus* spp. and *Urochloa* spp.

Subgroups	Studies <sup>@</sup>	N <sup>#</sup>	MD (95% CI) <sup><math>\pounds</math></sup> Random effect	P-value	Heterogeneity <sup>\$</sup> I <sup>2</sup> (%)	P-valu
Forage accumulation (Mg	/ha)				- ()	
Distance between tree row	(na)					
Eu1-14 <sup>&amp;</sup>	2 <sup>(22 and 27)</sup>	14 <sup>(54)</sup>	-2.76 [-3.65; -1.87]	< 0.001	87.6	< 0.00
Eu15-28	3 <sup>(6, 24 and 27)</sup>	16 <sup>(156)</sup>	-0.853 [-1.08; -0.624]	0.004	98.1	< 0.00
More28m*	7 <sup>(5,6,8,11,15,16</sup> and 28)	22 <sup>(85)</sup>	-0.331 [-1.40; 0.741]	0.126	97.4	< 0.0
Tree population						
Up to 99	3 <sup>(5,8 and 16)</sup>	5 <sup>(20)</sup>	4.06 [1.36; 6.76]	< 0.001	97.1	< 0.0
100-199	3 <sup>(5,11 and 15)</sup>	9 <sup>(36)</sup>	-1.83 [-2.67; 0.192]	0.256	88.6	< 0.0
200-299	5 <sup>(8,16,18,22 and 28)</sup>	10 <sup>(39)</sup>	-0.602 [-1.46; 0.253]	0.458	96.4	< 0.0
300-399	2 <sup>(6 and 24)</sup>	14(150)	-0.626 [-0.834; -0.419]	< 0.001	97.9	< 0.0
400-499	2 <sup>(22 and 27)</sup>	6(22)	-2.75 [-4.36; -1.14]	< 0.001	91.4	< 0.0
More than 499	3(6, 22 and 27)	8 <sup>(28)</sup>	-4.87 [-7.30; -2.45]	< 0.001	96.7	< 0.0
Tree row orientation						
East-west	9 <sup>(5,6,8,11,16,18,22,24 and 29)</sup>	44 <sup>(267)</sup>	-0.791 [-1.00; -0.579]	0.036	97.3	< 0.0
North-South	2 <sup>(15 and 27)</sup>	8(28)	-3.23 [-5.05; -1.41]	< 0.001	94.5	< 0.0
Forage species			,			
Marandu	6 <sup>(5,8,11,16,18 and 28)</sup>	16 <sup>(63)</sup>	1.06 [-0.027; 2.14]	0.329	97.1	< 0.0
Piatã	3(6,24 and 27)	20 <sup>(168)</sup>	-1.28 [-1.53; -1.04]	0.019	98.2	< 0.0
Decumbens	2(15 and 22)	16 <sup>(64)</sup>	-2.59 [-3.25; -1.93]	0.009	80.4	< 0.0
System age	-	10	2105 [ 0120, 1150]	0.000	0011	0.01
Four-five	8(5,6,8,16,18,22,24 and 27)	43 <sup>(259)</sup>	-0.959 [-1.18; -0.734]	< 0.001	97.5	< 0.0
Six-seven	2 <sup>(11</sup> and 28)	5 <sup>(20)</sup>	-0.939 [-1.16, -0.734]	0.563	95.1	< 0.0
More than seven	1 <sup>(15)</sup>	4 <sup>(16)</sup>	-2.80 [-4.76; -0.835]	< 0.001	92.1	< 0.0
Forage density (kg DM/ha		т	-2.00 [-4.70, -0.000]	< 0.001	92.1	< 0.0
Distance between tree row	4 oney					
Eu1-14 <sup>&amp;</sup>	$1^{(26)}$	2 <sup>(6)</sup>	E6 0 [ 60 4; E1 6]	< 0.001	0.00	0.320
Eu15-28	1 1 <sup>(26)</sup>	$2^{(6)}$	-56.0 [-60.4; -51.6] -36.8 [-46.3; -27.3]	< 0.001	64.0	0.320
More28m*	7(5, 8, 11, 13, 14, 19 and 28)	2 21 <sup>(78)</sup>	-20.1 [-24.8; -15.4]	< 0.001	87.8	< 0.0
	/	21	-20.1 [-24.8, -15.4]	< 0.001	07.0	< 0.0
Tree population	2 <sup>(5 and 8)</sup>	$3^{(12)}$	0.05 [ 16.0, 1.22]	0.020	65.9	0.050
Up to 99	5 <sup>(5, 8, 11, 13</sup> and 14)	10 <sup>(38)</sup>	-9.05 [-16.8; -1.32]	0.020		
100-199	2 <sup>(8 and 28)</sup>	8 <sup>(28)</sup>	-19.9 [-26.0; -13.8]	< 0.001	90.7	< 0.0
200-299	2 <sup>(26)</sup>	2 <sup>(6)</sup>	-26.2 [-33.9; -18.5]	< 0.001	65.9	0.005
400-499	1 <sup>(26)</sup>	2 <sup>(6)</sup>	-36.8 [-46.3; -27.3]	< 0.001	64.4	0.100
More than 499	1(10)	2(0)	-56.0 [-60.4; -51.6]	< 0.001	0.00	0.320
Tree row orientation	5 <sup>(5, 8, 11, 19 and 28)</sup>	<b>4</b> = (56)				
East-west	3(13, 14 and 26)	15 <sup>(56)</sup> 10 <sup>(34)</sup>	-19.1 [-24.3; -14.0]	< 0.001	83.7	< 0.0
North-South	3(10, 11 and 20)	10(01)	-32.4 [-43.5; -21.2]	< 0.001	96.5	< 0.0
Forage species	5 <sup>(5, 8, 11, 19 and 28)</sup>	<b>1</b> = (56)				
Marandu		15(56)	-19.1 [-24.3; -14.0]	< 0.001	83.7	< 0.0
Piata	$1^{(26)}$	4 <sup>(12)</sup>	-47.8 [-61.6; -34.0]	< 0.001	93.9	< 0.0
Decumbens	2 <sup>(13 and 14)</sup>	6 <sup>(22)</sup>	-22.1 [-32.8; -11.4]	< 0.001	93.3	< 0.0
Pasture height (cm)						
Distance between tree row	(2, 22, 22, 25, and 27)	(00)				
Eu1-14	5 <sup>(3, 22, 23, 25 and 27)</sup>	24 <sup>(98)</sup>	-7.82 [-11.4; -4.21]	< 0.001	100	< 0.00
Eu15-28	6 <sup>(3, 6, 9, 17, 23 and 27)</sup>	17(61)	3.61 [-0.113; 7.34]	0.060	100	< 0.00
More28m	3 <sup>(6, 9 and 9)</sup>	6 <sup>(27)</sup>	1.96 [0.850; 3.07]	< 0.001	9.70	0.350
Forage species						
Marandu	2 <sup>(12 and 25)</sup>	3(24)	-15.1 [-40.7; 10.6]	0.250	90.0	< 0.0
Piatã	6 <sup>(3, 6, 9, 17, 23 and 27)</sup>	32 <sup>(114)</sup>	1.27 [-1.39; 3.92]	0.310	100	< 0.0
Decumbens	1 <sup>(22)</sup>	12 <sup>(48)</sup>	-10.5 [-15.0; -6.06]	< 0.001	84.9	< 0.0
Leaf area index						
Distance between tree row						
Eu1-14	1 <sup>(27)</sup>	2 <sup>(6)</sup>	-0.954 [-1.13; -0.781]	< 0.001	0.00	0.340
Eu15-28	2 <sup>(6 and 27)</sup>	4(12)	-0.587 [-0.930; -0.245]	< 0.001	70.8	0.020
More28m	5 <sup>(6, 10, 11, 12 and 19)</sup>	13(102)	0.174 [-0.060; 0.408]	0.140	87.7	< 0.0
Tree population						
100-199	3 <sup>(10, 11 and 12)</sup>	7 <sup>(84)</sup>	0.629 [0.530; 0.729]	< 0.001	29.8	0.200
200-299	1 <sup>(19)</sup>	4 <sup>(12)</sup>	-0.025 [-0.352; 0.302]	0.880	26.6	0.250
300-399	1 <sup>(6)</sup>	2 <sup>(6)</sup>	-0.710 [-1.20; -0.220]	0.005	56.7	0.130
100-499	1 <sup>(27)</sup>	2 <sup>(6)</sup>	-0.400 [-0.603; -0.197]	< 0.001	0.00	0.999
More than 499	2 <sup>(6 and 27)</sup>	4 <sup>(12)</sup>	-0.883 [-1.15; -0.617]	< 0.001	56.2	0.080
Tree row orientation						
East-west	5(6, 10, 11, 12 and 19)	15(108)	0.023 [-0.244; 0.291]	0.860	91.8	< 0.0
North-South	1 <sup>(27)</sup>	4 <sup>(12)</sup>	-0.659 [-0.998; -0.321]	< 0.001	82.8	< 0.0
Forage species	-		0.009 [ 0.990, -0.021]	0.001	02.0	< 0.0
Marandu	4 <sup>(10, 11, 12 and 19)</sup>	11 <sup>(96)</sup>	0.414 [0.245; 0.583]	< 0.001	70.1	< 0.0
Piatã	2 <sup>(6 and 27)</sup>	8 <sup>(24)</sup>	-0.707 [-0.942; -0.472]	< 0.001	70.1 72.7	< 0.0 < 0.0
	2	0	-0.707 [-0.774, -0.472]	< 0.001	1 4.1	< 0.0
System age	3(6, 19 and 27)	12 <sup>(36)</sup>	-0.513 [-0.757; -0.269]	< 0.001	78.3	< 0.0
Zour five						< 0.0
Four-five Six-even	2 <sup>(10 and 11)</sup>	6 <sup>(72)</sup>	0.626 [0.516; 0.735]	< 0.001	40.8	0.130

Distance between tree row

(continued on next page)

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#### Table 4 (continued)

Subgroups	Studies <sup>@</sup>	$\mathbf{N}^{\#}$	MD (95% CI) <sup>£</sup> Random effect	P-value	Heterogeneit I <sup>2</sup> (%)	y <sup>\$</sup> P-value
Eu1-14	1 <sup>(27)</sup>	2 <sup>(6)</sup>	39.3 [-0.063; 78.7]	0.050	98.8	< 0.001
Eu15-28	1 <sup>(27)</sup>	$2^{(6)}$	16.6 [-5.30; 38.6]	0.140	96.9	< 0.001
More28m	2 <sup>(10 and 11)</sup>	6 <sup>(72)</sup>	11.3 [6.4; 16.1]	< 0.001	77.2	< 0.001
Dead material percent	age (%)					
Distance between tree ro						
Eu1-14	1 <sup>(25)</sup>	2 <sup>(6)</sup>	2.11 [0.522; 3.80]	0.009	35.0	0.210
Eu15-28	2 <sup>(1 and 9)</sup>	5(17)	-2.85 [-5.48; -0.213]	0.030	97.7	< 0.001
More28m	6 <sup>(7, 9, 10, 11, 14 and 28)</sup>	19(121)	-3.78 [-5.47; -2.09]	< 0.001	92.9	< 0.001
Tree row orientation						
East-west	8 <sup>(1, 7, 8, 9, 10, 11, 25 and 28)</sup>	24 <sup>(143)</sup>	-2.31 [-3.38; -1.25]	< 0.001	94.3	< 0.001
North-South	1 <sup>(14)</sup>	2 <sup>(8)</sup>	-6.70 [-15.3; 1.92]	0.130	94.2	< 0.001
Forage species						
Marandu	7 <sup>(1, 7, 8, 10, 11, 25 and 28)</sup>	18(124)	-3.48 [-5.63; -1.32]	0.002	91.8	< 0.001
Piatã	1 <sup>(9)</sup>	6 <sup>(18)</sup>	-1.52 [-2.79; -0.248]	0.020	96.6	< 0.001
Decumbens	1 <sup>(14)</sup>	2 <sup>(8)</sup>	-6.7 [-15.3; 1.92]	0.130	94.2	< 0.001
Tiller number (tiller/n	1 <sup>2</sup> )					
Tree population						
100-199	3 <sup>(11, 13 and 15)</sup>	9 <sup>(34)</sup>	-332 [-483; -180]	< 0.001	93.8	< 0.001
200-299	2 <sup>(19 and 25)</sup>	6 <sup>(24)</sup>	-102 [-156; -48.2]	< 0.001	37.2	0.160
System age						
Four-five	1(19)	4 <sup>(12)</sup>	-80.3 [-137; -23.0]	0.006	32.2	0.220
Six-seven	1(11)	3(12)	-630 [-831; -429]	< 0.001	74.7	0.020
More than seven	3 <sup>(13, 15 and 25)</sup>	8 <sup>(34)</sup>	-190 [-295; -84.8]	< 0.001	87.8	< 0.001

<sup>@</sup>, number of studies in each subgroup (superscript numbers refer to list of papers listed in supplementary file 1)

<sup>#</sup>, number of comparisons in each subgroup (superscript numbers refer to number of samples in each subgroup)

 $^{\pounds}$  , mean differences between grass monoculture and silvopastoral systems

 $I^2$  = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for  $\chi^2$  (Q) test of heterogeneity

 $^{\&}$ , Eu1-14 = eucalyptus with 1 to 14m between tree rows

 $\urcorner$  , Eu15-28 = eucalyptus with 15 to 28m between tree rows

<sup>\*</sup>, More28m = eucalyptus with more than 28m between tree rows

 $\hat{}$  = the comparisons in Eu1-14 are equal to more than 499 trees, the comparisons in Eu15-28 are equal to 400 to 499 trees and the comparison in More28m are equal to 100 to 199 trees.

#### Table 5

Subgroup analysis for forage nutritional value variables according to tree population, distance between tree rows, tree row orientation, system age and forage species in silvopastoral system with *Eucalyptus* spp. and *Urochloa* spp.

Subgroups	Studies <sup>@</sup>	N <sup>#</sup>	MD (95% CI) <sup>£</sup>		Hetero	geneity <sup>\$</sup>
			Random	P-	$I^2$	P-
			effect	value	(%)	value
Crude protein	(% DM)					
System age						
Up to three	$1^{(21)}$	8(32)	0.774	0.04	93.5	<
			[0.028;			0.001
			1.52]			
Four-five	4 <sup>(9, 22, 24</sup>	30(174)	1.71 [0.916;	<	97.9	<
	and 26)		2.50]	0.001		0.001
Six-seven	3 <sup>(3, 17 and</sup>	$12^{(48)}$	1.66 [0.844;	<	94.7	<
	28)		2.47]	0.001		0.001
More than	3 <sup>(12, 14 and</sup>	9 <sup>(44)</sup>	3.38 [2.62;	<	99.7	<
seven	23)		4.15]	0.001		0.001
In vitro dry m	atter digestibil	ity (% DM	)			
Tree row orien						
East-west	1 <sup>(9)</sup>	6 <sup>(18)</sup>	1.21 [-1.04;	0.29	65.4	0.01
			3.46]			
North-South	3 <sup>(14, 26 and</sup>	7 <sup>(23)</sup>	-1.62 [-3.24;	0.05	55.2	0.04
	29)		-0.005]			

<sup>@</sup>, number of studies in each subgroup (superscript numbers refer to list of papers listed in supplementary file 1)

<sup>#</sup>, number of comparisons in each subgroup (superscript numbers refer to number of samples in each subgroup)

 ${}^{\pounds}$  , mean differences between grass monoculture and silvopastoral systems

 $I^{2}$  = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for  $\chi 2$  (Q) test of heterogeneity.

Egger's regression, probably because 65% of the comparisons were performed in SPSs with less than 28m between tree rows, which generates a large number of true negative effects. However, this reduction in FM is a normal physiological behavior of tropical grasses under shade and does not represent a real publication or article selection bias.

One of the main factors that affects FM and FA in SPSs is the amount of photosynthetically active radiation that reaches the pasture, which determines plant photosynthetic rate (Guenni et al., 2008; Santiago--Hernández et al., 2016). The results showed that tree populations of up to 99 trees/ha, spacing between tree rows greater than 28m and tree rows with an east-west orientation are recommendations that should be used when planning cattle production systems. The utilization of smaller tree populations in SPSs with greater spacing between tree rows is a strategy that has been used to increase the amount of light reaching the pasture and maintain photosynthetic rate (Magalhães et al., 2018; Domiciano et al., 2020).

Tree rows with an east-west orientation allows shade to be concentrated close to the tree trunk and minimizes the reduction of FM compared to trees row with a north-south orientation. This difference was observed for *U. brizantha* cv. Piatã in SPSs in the Brazilian Cerrado region with *Eucalyptus* spp. with 22m between tree rows, with the greatest reduction in FM being with tree rows with a north-south orientation (11.7 vs. 5.77 Mg/ha; 50.5% reduction) in the Santos et al. (2016) study compared to tree rows with an east-west orientation (7.27 vs. 4.78 Mg/ha; 34.3% reduction) in the study by Oliveira et al. (2014). Therefore, the results showed that *Eucalyptus* spp. rows with an east-west orientation is an important recommendation to maintain FM and FA. However, it is important to emphasize that in regions with slopes, tree rows should be established in an orientation opposite to the slope to reduce soil erosion (Paciullo et al., 2009; Lima et al., 2018).

FM was lower in SPSs of all ages, compared to grass monoculture, with no further reduction being observed in older systems. This high reduction was expected because the greater growth of aboveground tree biomass increases shading (Oliveira et al., 2015; Lima et al., 2019). This result was due to the tendency of pruning trees in older systems, which was seen in the greater tree populations with up to three years of age (average of 600 trees/ha) compared to those with more than seven years

#### Table 6

Subgroup analysis for animal performance variables according to tree population, distance between tree rows, tree row orientation, system age and forage species in silvopastoral system with *Eucalyptus* spp. and *Urochloa* spp.

Subgroups	Studies <sup>@</sup>	N <sup>#</sup>	MD (95% CI) <sup>f</sup> Random	Р-	Hetero I <sup>2</sup>	geneity <sup>\$</sup> P-
			effect	value	(%)	value
Stocking rate	(AU/ha)					
Distance betwe		(07)				
Eu1-14 <sup>&amp;</sup>	3 <sup>(21, 23 and 25)</sup>	7 <sup>(27)</sup>	-0.774	<	98.5	<
			[-1.06;	0.001		0.001
Eu15-28*	6 <sup>(1, 17, 20, 21,</sup>	14 <sup>(55)</sup>	-0.482] -0.342	<	98.2	<
Lu10-20	23 and 26)	14	[-0.535;	0.001	90.2	0.001
			-0.149]			
More28m	6 <sup>(5, 7, 8, 13, 16</sup>	$13^{(51)}$	0.164	0.150	93.0	<
	and 28)		[-0.061;			0.001
m 1.:			0.389]			
Tree population Up to 99	3 <sup>(5, 8 and 16)</sup>	4 <sup>(16)</sup>	0.637	<	18.4	0.300
001000	5	т	[0.534;	0.001	10.4	0.500
			0.739]			
100-199	4 <sup>(1, 5, 13 and</sup>	4 <sup>(15)</sup>	-0.105	0.350	79.6	0.002
	20)		[-0.327;			
	7 <sup>(7, 8, 16, 17,</sup>	(64)	0.117]			
200-299	21, 23 and 28)	16 <sup>(64)</sup>	-0.181	0.030	97.6	<
			[-0.345; -0.017]			0.001
300-399	2 <sup>(21 and 23)</sup>	6(24)	-0.691	<	98.7	<
			[-1.00;	0.001		0.001
			-0.382]			
400-499	3 <sup>(1, 20 and 26)</sup>	$3^{(11)}$	-0.660	0.004	87.3	<
			[-1.11;			0.001
Tree row orien	tation		-0.212]			
East-west	10 <sup>(1, 5, 7, 8,</sup>	31(124)	-0.176	0.020	98.5	<
Lust West	16, 17, 20, 21,	01	[-0.326;	01020	5010	0.001
	23 and 28)		-0.027]			
North-	2 <sup>(13 and 26)</sup>	3 <sup>(9)</sup>	-0.793	0.060	98.4	<
South			[-1.63;			0.001
Foraça maciac			0.043]			
Forage species Marandu	7 <sup>(1, 5, 7, 8, 16,</sup>	16 <sup>(64)</sup>	0.069	0.590	95.8	<
Maranda	20 and 28)	10	[-0.184;	0.050	50.0	0.001
			0.322]			
Piatã	4 <sup>(17, 21, 23 and</sup>	17 <sup>(66)</sup>	-0.535	<	99.0	<
	26)		[-0.735;	0.001		0.001
Crustom and			-0.335]			
System age Up to three	1 <sup>(21)</sup>	8(32)	-0.362	0.010	99.4	<
op to thee	1	0	[-0.644;	0.010	<i></i>	0.001
			-0.079]			
Four-five	7 <sup>(1, 5, 7, 8, 16,</sup>	16 <sup>(62)</sup>	-0.098	0.520	97.1	<
	20 and 26)		[-0.393;			0.001
<i>c</i> :	2 <sup>(17 and 28)</sup>	4(16)	0.198]	0.000	=1.0	0.010
Six-seven	Z(-)	4()	-0.003 [-0.219;	0.980	71.8	0.010
			0.213]			
More than	2(13 and 23)	6 <sup>(23)</sup>	-0.804	0.010	83.5	<
seven			[-1.42;			0.001
			-0.185]			
	gain (kg/anima	l/day)				
Tree population Up to 99	3 <sup>(5, 8 and 16)</sup>	5 <sup>(60)</sup>	0.148	<	81.7	<
50 10 55	5	0	[0.061;	0.001	01./	0.001
			0.236]			
100-199	4 <sup>(1, 5, 13 and</sup>	6 <sup>(86)</sup>	-0.021	0.400	88.3	<
	20)		[-0.068;			0.001
000 000	6 <sup>(7, 8, 16, 23</sup>	1 (86)	0.027]	0.010	( ) ?	
200-299	6 <sup>(7, 8, 10, 23</sup> and 28)	$16^{(86)}$	-0.024	0.310	64.2	<
	-		[-0.070; 0.022]			0.001
300-399	2 <sup>(21 and 23)</sup>	6 <sup>(24)</sup>	0.009	0.870	85.4	<
		-	[-0.106;			0.001
	0.00		0.125]			
400-499	3 <sup>(1, 20 and 26)</sup>	$3^{(50)}$	-0.048	<	28.3	0.200
			[-0.064;	0.001		
			-0.033]			

<sup>@</sup>, number of studies in each subgroup (superscript numbers refer to list of papers listed in supplementary file 1)

<sup>#</sup>, number of comparisons in each subgroup (superscript numbers refer to number of samples in each subgroup)

f, mean differences between grass monoculture and silvopastoral systems

 $\ ^{\$}\ I^2 =$  proportion of total variation of size effect estimates that is due to heterogeneity, P-value for  $\chi 2$  (Q) test of heterogeneity

 $\ensuremath{^\&}$  , Eu1-14 = eucalyptus with 1 to 14m between tree rows

 $^{*}$ , Eu15-28 = eucalyptus with 15 to 28m between tree rows

, More28m = eucalyptus with more than 28m between tree rows.

since tree planting (224 trees/ha). Furthermore, although studies have shown that *U. decumbens* is more resistant to shading (Guenni et al., 2008; Gobbi et al., 2009), the results of the present study showed *U. brizantha* cv. Marandu to have the highest forage mass and accumulation in SPSs with *Eucalyptus* spp., and so planting this cultivar should be used as a strategy to mitigate the negative effects of light restriction.

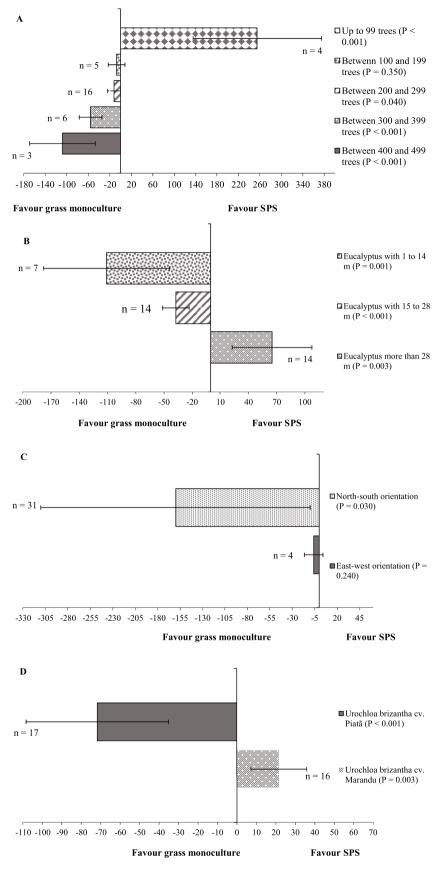
Forage density was reduced in all subgroups, with greater reductions with smaller spacing between tree rows, larger tree populations, tree rows with a north-south orientation and with *U. brizantha* cv. Piatā. These results are mainly explained by the reduction in FM and confirm the need to use smaller tree populations to provide adequate forage for the animals (Crestani et al., 2017; Santos et al., 2018). Pasture height was lower in SPSs with 1 to 14m between tree rows, but greater in SPSs with more than 28m. The lower pasture height with lower tree row spacing was probably due to severe reduction in luminosity and plant growth limitation. Plants in systems with greater spacing and less light restriction present greater height as a morphological change that allows them to reach higher pasture strata and capture more light (Geremia et al., 2018; Lima et al., 2019).

Plants under moderate shading show morphological changes that allow them to increase light capture (Gómez et al., 2012; Taiz et al., 2015; Paciullo et al., 2016). The present study found that even though the leaf area index was lower with up to 28m spacing between tree rows and with more than 299 trees, specific leaf area was greater in the SPSs. Increased specific leaf area is an alteration that allows a plant to increase its leaf surface and capture more light. Another change observed was the reduction of PPSM with greater than 15m between tree rows. Plant senescence can be triggered by environmental stresses, such as high temperatures and radiation, which explains the reduction in PPSM in SPSs (Taiz et al., 2015; Guenni et al., 2018). Tiller number was also lower in all SPSs, compared to grass monoculture, which partially explains the reduction in FM and forage density and shows the negative effect of shading on pasture vigor (Araújo et al., 2020). The results showed that although plants present morphological alterations that allow them to capture more light, these alterations were not enough to compensate for the light restriction of denser systems with less than 28m between tree rows or more than 199 trees/ha.

#### 4.3. Nutritional value

CP was greater in all SPSs, compared to grass monoculture, independent of the spacing between tree rows and tree population. This is an important result because it shows that in the systems with smaller tree populations, where FM was similar to or greater than for grass monoculture, moderate shading also increases pasture CP. Therefore, in these conditions, forage is offered in similar or greater quantity and with greater CP than in grass monoculture, which might have contributed to generating greater GHA and may indicate lower need for CP supplementation. Furthermore, the increase in CP in all SPSs, compared to grass monoculture, created an asymmetry in Egger's regression due to the high occurrence of positive effects.

Increases in CP concentration in shaded environments are due to increased soil nitrogen (N), which is probably explained by it being an environment that favors increased mineralization and availability of soil N and by increased litter deposition and reduced soil disturbance, both



**Fig. 4.** Subgroup analysis for total weight gain per area (kg/ha) according to trees population (A), distance between rows (B), row orientation (C) and forage species (D) in silvopastoral system with *Eucalyptus* spp. and *Urochloa* spp. N, indicates the number of comparisons in each subgroup.

of which increase the supply of soil N (Wilson, 1996; Baah-Acheamfour et al., 2015). Another explanation is lower plant N dilution in SPSs due to lower FA. According to nitrogen dilution theory, there is an optimal percentage of nitrogen for a given level of forage production. In grass monoculture, there is greater forage production and translocation of absorbed N to other parts of the plant, which dilutes N. On the other hand, in shaded plants, where there is less forage production, all absorbed N is not metabolized and is not converted into forage accumulation, which explains the greater N accumulation (Lemaire and Chartier, 1992; Kephart and Buxton, 1993). In addition, shaded plants remain at a younger physiological stage (Taiz et al., 2015), which reduces fiber accumulation (Paciullo et al., 2007; Sousa et al., 2010) and proportionally increases CP. This maintenance of physiological stage was confirmed by the lower PPSM in SPSs.

Neutral detergent fiber reduction is mainly due to the maintenance of plant cells at a younger physiological stage, which reduces the accumulation of this fraction (Taiz et al., 2015). This explanation was confirmed by the lower PPSM and the greater CP concentration in SPSs, which are traits of younger forages. Deinum et al. (1996) observed that *U. brizantha* under low light incidence had less sclerenchyma cells (supporting cells) and thinner cell walls compared to plants subjected to high light intensity. These cells with thinner cell walls can generate thinner leaves and, thus, have reduced fiber concentration, as observed in the present study (Gómez et al., 2012). Although plants in SPSs showed less neutral detergent fiber and greater CP, compared to grass monoculture, equal IVDMD and increased lignin indicate no significant increase in nutritive value. Therefore, the increase in GHA in systems with lower tree density was probably due to an increase in forage accumulation and not to a reduction in NDF and increase in CP.

Lignin concentration was greater in SPSs compared to grass monoculture. In some specific subgroups, such as those with more than 28m between tree rows, plants were taller. This greater plant height in SPSs is a morphological alteration that allows a plant to reach upper pasture strata (Paciullo et al., 2016; Santos et al., 2016), which was corroborated by the increase in percentage of plant stem observed in the present study. This greater pasture height and percentage of plant stem in some specific subgroups may explain the increase in lignin because the stem is highly lignified to support the weight of the plant. This increase in lignin was not able to reduce IVDMD in SPSs, probably due to the increase in CP and reduction in neutral detergent fiber. This result is important because it shows that even if plants have greater lignin in SPSs, animal intake is probably not altered by physical limitation because there is no reduction in forage IVDMD (Allen, 1996; Mertens and Grant, 2020).

The opposite behavior of increasing lignin concentration and decreasing NDF concentration is not an expected result. The great difference between the concentrations of NDF and lignin in the leaf and stem is probably related to this behavior. The lower metabolic rate and stress that occurs in leaves, which reduce accumulation of NDF, and the lower proportion of senescent material in plants in SPSs compared to grass monoculture, may explain the reduction in NDF. However, many studies have shown an increase in plant height and percentage plant stem, which explains the increase in lignin. All the data used in the present study came from the chemical composition of the whole plant, which is influenced by both leaf and stem, and the combination of these phenomena probably explains the reduction in NDF and increase in lignin. Importantly, the comparisons used in different subgroups for different variables were not always from exactly the same studies, as the studies did not assess the same variables. Thus, these different comparisons from different studies may explain some inconsistent results.

#### 4.4. Animal performance

One of the main parameters regarding beef cattle system performance is GHA because it is directly related to profitability. In the present study, average daily gain was similar between systems, and so the main variable that affected GHA was stocking rate (Santos et al., 2018; Domiciano et al., 2020; Pereira et al., 2021). Pasture stocking rate is mainly influenced by FM and FA and the results of the present study showed that FM, FA, SR and GHA were markedly reduced with large tree populations. This result is important because for SPSs to be implemented in commercial farms they need to generate a financial result similar or superior to that of grass monoculture. In addition, it is important to note that pasture nutritive value was not significantly improved in SPSs compared to grass monoculture, which indicates that FM and FA were the main factors that alter GHA in SPSs.

The results showed that with spacing greater than 28m between tree rows and up to 99 trees/ha allows greater GHA, which, together with the wood production from *Eucalyptus* spp., can result in greater profitability and facilitate SPS implementation. Furthermore, using 100 to 299 trees/ ha produced GHA very similar to that of grass monoculture. These results indicate that these tree populations should be considered during SPS planning because the increase in profitability from wood can increase total system profitability (Domiciano et al., 2016; Pontes et al., 2021), even with slight GHA reduction. The balance of tree population is essential so as not to reduce pasture productivity and maximize animal and wood production, thereby increasing profitability per area with livestock.

GHA was lower in SPSs with a north-south orientation compared to grass monoculture, but it did not differ for those with an east-west orientation. These results were similar to those for FM and FA and indicate that shading is more accentuated with north-south tree row arrangements. Another important result of the present study was the superior performance of *U. brizantha* cv. Marandu for SR and GHA compared to the other forage species, which indicates that this forage is suitable for use in SPSs. However, there was a low number of comparisons for *U. brizantha* cv. Piatã, and so it needs further evaluation. An insufficient number of comparisons with *U. decumbens* were found to perform subgroup analysis with this forage. Some studies have indicated that this forage has greater shade tolerance (Guenni et al., 2008; Gobbi et al., 2009), and so more studies on animal performance in SPSs with this forage are also needed.

The covariates explained approximately only 50% of the variation in the data. This low percentage indicates that there are other factors that affect *Urochloa* spp. traits and animal performance, which explains the high heterogeneity found. It is possible that this low explanation was generated by competition between trees and pasture for water and nutrients inside SPSs or by the evaluations being done in different climatic seasons, at different places in the rank or with different distances from the tree row. These factors directly influence FM and forage quality and alter animal performance. In addition, animal-related factors, such as category and breed, influence performance. Nonetheless, it was decided that only the effect of distance between tree rows, tree population, tree row orientation, system age and forage species be evaluated because these factors can be controlled during SPS implementation and were well described in all studies.

#### 5. Conclusion

Most publications that met the inclusion criteria and were used in the present study had a duration of less than one year, which indicates the need to develop studies of longer durations to better understand how changes in competition for light, nutrients and water over time affect pasture and animal performance in silvopastoral systems. Pasture in silvopastoral systems established with *Eucalyptus* spp. and *Urochloa* spp. showed less neutral detergent fiber and greater crude protein concentrations than did grass monoculture, yet they also showed greater lignin and no difference for *in vitro* dry matter digestibility, which indicates no improvement in pasture nutritive value. Forage mass was reduced in silvopastoral systems with more than 99 trees/ha or less than 28m between tree rows. The utilization of less than 99 trees/ha, greater than 28m between tree rows, tree rows with an east-west orientation and *Urochloa brizantha* cv. Marandu are recommendations that can be used

to maximize *Urochloa* spp. and beef cattle performance, thereby increasing total system production and facilitating the implementation of silvopastoral systems in commercial farms.

#### Credit author statement

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2022.104973.

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## 1 CAPÍTULO 4: ARTIGO - EFFECTS OF SHADING ON TROPICAL GRASS

## 2 CHARACTERISTICS AND CATTLE PERFORMANCE IN SILVOPASTORAL

## 3 SYSTEMS: SYSTEMATIC REVIEW AND META-ANALYSIS

## 4 Artigo publicado na Animal Production Science (Impact Factor 1.57)



RESEAR CH PAPER https://doi.org/10.1071/AN22313 ANIMAL PRODUCTION SCIENCE

## Effects of shading on tropical grass characteristics and cattle performance in silvopastoral systems: systematic review and meta-analysis

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© 2023 The Author(s) (or their employer(s)). Published by CSIRO Publishing. ABSTRACT

Context. Shading reduces forage mass and can reduce animal production and profitability per area in silvopastoral systems (SPSs) with tropical grasses. This reduction in profitability is the main obstacle to using such systems. Aims. This study evaluated the effects of shading by different tree arrangements on tropical grass characteristics and cattle performance in SPSs. Methods. Systematic searches were conducted in databases and directly in scientific journals, and 66 articles were selected. Data were grouped into SPS subgroups on the basis of tree type: with Eucalyptus with 1-14 m between rows; with Eucalyptus with 15-28 m between rows; with Eucalyptus with more than 28 m between rows; with leguminous trees; with palm trees; and with other types of tree. Data were analysed with random-effects model by using mean difference with 95% confidence interval (at P = 0.05). Results. A large reduction in forage mass significantly reduced animal weight gain per area and stocking rate of beef cattle reared in SPSs with row spacing of up to 28 m, compared with pasture monoculture. There was a small reduction in forage mass in SPSs with Eucolyptus with more than 28 m between rows, compared with pasture monoculture, but no reduction in stocking rate. This result allowed an increase in weight gain per area and indicated the need to use more than 28 m between Eucalyptus rows in systems the main objective of which is animal production. There was also a small reduction in forage mass in leguminous tree SPSs, but weight gain per area was similar to that in pasture monoculture; the animals also had a higher dry-matter intake, crude protein intake and milk production in these SPSs. The tropical grasses in palm tree SPSs had a higher crude protein and a lower forage mass than did those in pasture monoculture, and no reduction in weight gain per area compared with those in pasture monoculture, which indicated the possibility of productive animal production together with palm trees. The SPSs with other types of tree had a higher weight gain per area than did pasture monoculture. This result indicated that the use of SPSs with native trees can integrate animal production with environmental preservation. Conclusions. The SPSs with Eucolyptus with more than 28 m between the rows or with other types of tree had a higher weight gain per area than did pasture monoculture, whereas leguminous and palm tree SPSs had a weight gain per area similar to that of pasture monoculture, which indicated that there was no significant negative effect of shading on livestock production. Implications. Silvopastoral systems with higher weight gain per leaf area than, or similar to that of pasture monoculture can increase the total system production and profitability (considering wood and animal productions), which is beneficial and may be a factor in motivating producers to adopt these SPSs on commercial

Keywords: agroforestry, Eucalyptus, integrated systems, Leucaena leococephala, Megathyrsus, photosynthetically active radiation, trees, Urochloa.



ANIMAL PRODUCTION SCIENCE

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**Keywords:** agroforestry, *Eucalyptus*, integrated systems, *Leucaena leococephala*, *Megathyrsus*, photosynthetically active radiation, trees, *Urochloa*.

## Introduction

Silvopastoral systems (SPSs) have been developed, improved and used in tropical regions as an alternative to traditional monoculture systems. This use has been causing a great change in agricultural systems by improving environmental indicators, but mainly by increasing the system revenue. For example, Magalhães et al. (2018) observed bodyweight gain per area of 366 kg/ha (927 vs 561 kg bodyweight/ ha.year) higher in beef cattle on SPSs with Eucalyptus than on pasture monoculture. This increase in animal production would add approximately US\$610/ha.year in gross income to the system. In dairy cattle, Hernández-Rodríguez (2019) observed milk production 2 L higher (11.9 vs 9.9 L/cow.day) in SPSs with Leucaena leucocephala, an increase of approximately US\$240/cow.year of gross income. Furthermore, these systems have the potential to provide income from the wood produced.

These systems use different arrangements of tree, pasture and animal, so as to obtain better productive and environmental indicators. These systems are more diversified and, if they are not implemented with excessive shade, may present performance superior to that of monocultures due to synergism among their components (Lemaire *et al.* 2014; Magalhães *et al.* 2018). Some purported benefits of SPSs are increased soil carbon stock (Almeida *et al.* 2021), improvement of pasture nutritional value (Paciullo *et al.* 2014), improvement of animal comfort (Giro *et al.* 2019) and increased animal and plant productivity (Magalhães *et al.* 2018). Therefore, the use of SPSs may improve livestock performance and environmental indicators through sustainable intensification.

In Brazil, SPSs are mainly formed by different arrangements of Eucalyptus sp. trees and Urochloa sp. grasses (Stape et al. 2010). Moderate shading allows forage to develop morphological changes to compensate for light reduction (Gómez et al. 2012; Paciullo et al. 2016). However, studies conducted in Brazil with high tree density (spacing between rows close to, or lower than, 20 m) showed that severe light restriction (a reduction of more than 50%) reduces pasture productivity and animal performance per unit area (Oliveira et al. 2014; Santos et al. 2016, 2018). At lower tree densities, some studies have shown only small reductions in pasture production and the maintenance of, or increases in, animal production (Magalhães et al. 2018; Carvalho et al. 2019; Domiciano et al. 2020). However, the results are inconsistent and there is no well defined recommendation for implementing these systems.

In other Latin American countries, such as Colombia and Mexico, SPSs are also important for increasing system performance (Murgueitio *et al.* 2011). These systems are more complex and generally integrate pastures with grasses, legumes, shrubs and trees for wood production. The intercropping of these components increases the total forage production of systems and can increase animal performance (Vizzotto *et al.* 2015). However, the major obstacle to implementing these systems is still adjusting the management of different forage species in the same location and allowing these species to be integrated with high productivity for long periods (Paciullo *et al.* 2009, 2021). In some tropical regions, there are also systems that use native tree and shrub resources, which allows the development of livestock production together with environmental preservation (Sousa *et al.* 2010).

Considering that SPSs can improve or worsen indicators of livestock production and that there is still no systematic review and meta-analysis that evaluates the performance of tropical grasses and cattle in SPSs in tropical regions, this type of study can provide relevant summarised information about these systems. The indication of tree arrangements intercropped with grasses for tropical climates with better pasture and animal performance may provide relevant information to motivate and expand the implementation of these systems on commercial farms. Therefore, this study aimed to evaluate the effects of shading from different SPS tree arrangements on tropical grass characteristics and the impact on beef and dairy cattle performance.

## Materials and methods

## **Protocol and registration**

The protocol of this systematic review was submitted to and approved by the Open Science Framework with the title 'Effect of shading on the tropical pastures and cattle performances in silvopastoral systems: Systematic review and meta-analysis' (https://doi.org/10.17605/OSF.IO/7R2PH). This study followed the recommendations proposed by the guideline Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Page *et al.* 2021).

## Study eligibility criteria

Randomised studies published in Portuguese, English or Spanish in scientific journals that evaluated the effects of natural shading of trees on tropical grasses and that answered the question of population, intervention, comparison, outcomes (PICO), were included, and were as follows (Thomas et al. 2019): population: tropical grass pasture; intervention: natural shading of trees on tropical grasses; comparison: pasture monoculture; primary outcomes: dry-matter intake (kg/animal.day), crude protein intake (g/animal.day), average daily gain (kg/animal.day), total gain per area (kg/ha), stocking rate [animal unit (AU)/ha], milk production (kg/cow.day); and secondary outcomes: dry matter (% natural matter; DM), crude protein (% DM), neutral detergent fibre (% DM), acid detergent fibre (% DM), lignin (% DM), in vitro DM digestibility (% DM), in situ DM digestibility (% DM), forage mass (kg/ha), forage density (kg DM/cm.ha), height (cm), tiller number (tillers/ha), leaf area index, specific leaf area (cm<sup>2</sup>/g), plant leaf percentage (%), plant stem percentage (%) and plant dead material percentage (%).

## Information source and data search

The search strategy for articles was systematically executed in the databases Scopus, Embase, Web of Science and MEDLINE-Pubmed, directly in scientific journals and in the references of articles selected by the systematic review. The terms used to characterise the POPULATION were 'tropical pasture', 'tropical forage', 'tropical grassland', 'full sun pasture', 'pasture', 'grassland', 'Paspalum', 'Megathyrsus', 'Panicum', 'Brachiaria', 'Urochloa', 'Cynodon', 'Andropogon', 'Digitaria', 'Chloris gayana', 'Hyparrhenia rufa', 'Cenchrus ciliaris', 'Melinis minutiflora' and 'Pennisetum'. The terms to characterise the INTERVENTION were 'silvopastoral systems', 'livestock–forest integration', 'integrated livestock–forest system', 'integrated systems', 'agroforestry', 'shaded pasture' and 'shade'. The last survey was conducted 17 June 2021.

In total, 1887 articles were selected from the surveyed databases and, after excluding duplicates, the titles and

abstracts of 1560 articles were extracted (Fig. 1). Reading of the titles and abstracts resulted in the selection of 171 articles for full-text reading, with participant type being the main reason for exclusion in this phase (564 articles). Reading of the full text resulted in the selection of 56 articles, with result type being the main reason for exclusion in this phase (52 articles). In total, 10 articles were included by manual search. In total, 66 studies were used in this analysis.

## Publication bias risk and study's methodological quality

The methodological quality of the studies was assessed using the guidelines proposed by ARRIVE 2.0 Animal Research: reporting of *in vivo* experiments (du Sert *et al.* 2020). The criteria used were: Study design (For each experiment, provide brief details of study design including: The groups being compared, including control groups. If no control group has been used, the rationale should be stated), Sample size a (Specify the exact number of experimental units allocated to each group, and the total number in each

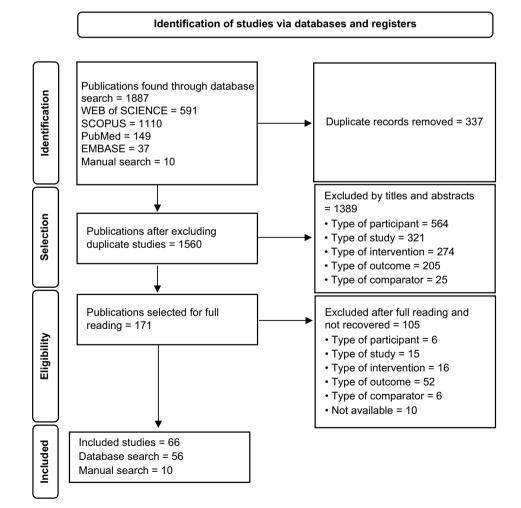


Fig. I. Flowchart of the selection process of articles included in the meta-analysis.

experiment. Also indicate the total number of animals used), Sample size b (Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done), Randomization (State whether randomization was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomization sequence), Outcome measures (Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioral changes), Statistical methods a (Provide details of the statistical methods used for each analysis, including software used), Statistical methods b (Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met), Experimental animals (Provide species-appropriate details of the animals used, including species, strain and sub strain, sex, age or developmental stage, and, if relevant, weight), Experimental procedures a (For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: What was done, how it was done and what was used), Experimental procedures b (When and how often.), Results (For each experiment conducted, including independent replications, report: Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and s.d., or median and range)), Protocol of animal use (Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered), Data access (Provide a statement describing if and where study data are available), Declaration of interests a (Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated) and Declaration of interests b (List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study) (Fig. S1; Supplementary material S1). Pairs of researchers performed the evaluations, with the help of a third in the case of disagreement. The risk of publication bias was assessed by testing the symmetry between the standard deviation and mean difference (MD) by using funnel plots (Higgins et al. 2019).

## Selection of articles and data-collection process

The articles were consolidated into a database after removing all duplicates with EndNote® article manager. Articles were extracted and peer reviewed using Microsoft Excel to record data. Prior training was conducted with three researchers from our research group to standardise the assessments. The following exclusion criteria were used: population type: studies conducted with temperate grass pastures or legumes; intervention type: studies that did not use natural shading by trees; study type: case studies, descriptive studies, nonprimary studies, patents, studies without control group(s) and studies without estimates of the mean and variation for the evaluated variables. Divergences in the opinions of researchers were solved with the help of a third researcher. Once articles were validated, data were extracted.

## Data synthesis and statistical analyses

Data analysis was performed using RevMan software version 5.4 (Cochrane Collaboration 2020). Data were analysed in six SPS subgroups based on tree type and spacing between rows, as follows: with *Eucalyptus* with 1–14 m between rows (Eu1–14 m); with *Eucalyptus* with 15–28 m between rows (Eu15–28 m); with *Eucalyptus* with more than 28 m between rows (Eu28 m); with leguminous trees; with palm trees; and with other types of tree. The tree species included in each subgroup are described in the Supplementary file S1.

When two or more eligible comparisons were selected within the same SPS subgroup, a meta-analysis was performed. Data were analysed with a random-effects model by using mean difference with 95% confidence interval (P < 0.05). Studies were weighted by the inverse of the variance. Heterogeneity ( $I^2$ ) was evaluated on the basis of the following criteria indicating low, moderate and high heterogeneity (P < 0.1) respectively:  $I^2$  lower than 30%,  $I^2$  between 31 and 75% and  $I^2$  greater than 75% (Deeks *et al.* 2019). Heterogeneity means that the confidence intervals for the results of individual studies overlap poorly, which generally indicates the presence of statistical heterogeneity.  $I_2$  describes the percentage of variability in the effect estimates due to heterogeneity rather than sampling error (chance).

## Results

## **Study characteristics**

The countries with the most articles were Brazil (53), Mexico (5) and Colombia (3). Eu1–14 m SPSs were used in 24.4% of the comparisons, leguminous tree SPSs in 22.8%, palm tree SPSs in 16.5%, Eu28 m SPSs in 13.9%, SPSs with other types of tree in 11.6% and Eu15–28 m SPSs in 10.9% (Table S1; Supplementary material S1). *Eucalyptus* SPSs, regardless of row spacing, accounted for 49.2% of the articles. The most used forage species were *U. decumbens* (26.4%), *U. brizantha* cv. Marandu (23.8%), *Megathyrsus maximus* (21.1%), *U. brizantha* cv. Piatã (19.1%), other grasses (4.3%), *Andropogon gayanus* (3.3%) and *Cynodon* sp. (2.0%).

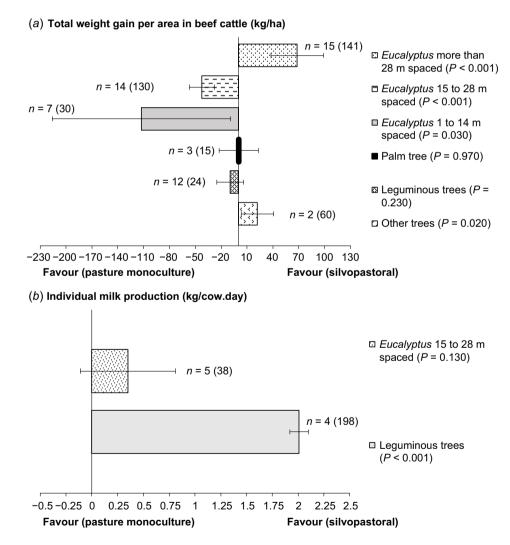
Considering the experimental periods of the 66 articles, 21 lasted up to 6 months, 32 lasted between 6 months and 1 year, nine lasted between 1 and 2 years and four lasted longer than 2 years. Thirty-three of the 66 articles evaluated some animal-performance variables, 25 with beef cattle and eight with dairy cattle. The animals in the beef cattle systems were predominantly of the Nellore breed and their crossings with other zebuine and taurine breeds. The dairy cattle were

predominantly of the Holstein breed and their crossings with zebuine dairy breeds.

#### Animal performance

Beef cattle weight gain per area was lower in Eu1–14 m (MD = -113; P = 0.03) and Eu15–28 m (MD = -42.4; P < 0.001) than in pasture monoculture (Fig. 2), and higher in Eu28 m (MD = 68.3; P < 0.001). Weight gain per area was not altered (P > 0.05) in SPSs with leguminous and with palm trees and was higher for SPSs with other types of tree (MD = 22.0; P = 0.02) than in pasture monoculture. Milk production was evaluated in only two SPS subgroups and was not influenced by Eu15–28 m (P = 0.13) and was higher in leguminous trees (MD = 1.14; P < 0.001), than in pasture monoculture.

Average daily gain was not influenced by any SPS subgroup (P > 0.05; Table 1). Only Eu1–14 m did not show high and significant heterogeneity (P < 0.01). Stocking rate was lower in Eu1–14 m (MD = -0.78; P < 0.001) and Eu15–28 m (MD = -0.24; P = 0.009), than in pasture monoculture, while it was similar between Eu28 m and pasture monoculture (P = 0.14). Stocking rate was lower in leguminous trees (MD = -0.20; P = 0.01) than in pasture monoculture, but similar in SPSs with other types of tree (P = 0.25) to that in pasture monoculture (Table 1). Heterogeneity was high in all eucalyptus subgroups (P < 0.001) and moderate in leguminous trees. Dry-matter and crude protein intakes were higher in SPSs with leguminous trees than in pasture monoculture (P < 0.01) and similar in the other SPSs subgroups to those in pasture monoculture.



**Fig. 2.** Mean difference for (a) total weight gain per area in beef cattle (kg/ha) and (b) individual milk production (kg/cow.day) in silvopastoral systems with tropical grasses according to tree types. *n* following each bar on the graph indicates the number of comparisons in each subgroup and the number inside parentheses indicates the number of animals.

Subgroup	Number of studies <sup>A</sup>	N <sup>B</sup>	MD (95% CI)	с	Heter	ogeneity
			Random effect	P-value	l <sup>2</sup> (%)	P-value
Average daily gain (g	;/animal.day)					
Eul-I4 m	<b>3</b> (44, 50 and 57)	7 <sup>(30)</sup>	-0.01 [-0.04, 0.01]	0.250	15	0.310
Eu15–28 m	6 <sup>(2, 37, 43, 44, 50 and 57)</sup>	I4 <sup>(130)</sup>	-0.02 [-0.07, 0.02]	0.370	85	<0.001
Eu28 m	6 <sup>(18, 20, 21, 31, 34 and 61)</sup>	I7 <sup>(164)</sup>	0.03 [-0.02, 0.09]	0.270	87	<0.001
Legume	l <sup>(62)</sup>	I 2 <sup>(24)</sup>	-0.02 [-0.23, 0.20]	0.860	92	<0.001
Palm	3 <sup>(3 and 22)</sup>	6 <sup>(37)</sup>	-0.09 [-0.27, 0.09]	0.320	74	0.002
Other trees	2 <sup>(35)</sup>	3 <sup>(68)</sup>	0.13 [-0.04, 0.30]	0.140	97	<0.001
Stocking rate (AU/h	a) <sup>D</sup>					
Eul-I4 m	<b>3</b> (44, 50 and 57)	7 <sup>(35)</sup>	-0.78 [-1.08, -0.48]	<0.001	99	<0.001
Eu15–28 m	<b>6</b> <sup>(2, 37, 44, 46, 50 and 57)</sup>	I 5 <sup>(75)</sup>	-0.24 [-0.42, -0.06]	0.009	98	<0.001
Eu28 m	6 <sup>(18, 20, 21, 31, 34 and 61)</sup>	I 4 <sup>(55)</sup>	0.16 [-0.05, 0.38]	0.140	95	<0.001
Legume	2 <sup>(39 and 61)</sup>	32 <sup>(144)</sup>	-0.20 [-0.35, -0.04]	0.010	48	0.001
Other trees	<sup>(35)</sup>	2(6)	0.42 [-0.30, 1.15]	0.250	0	0.990
Dry-matter intake (l	<pre> dg DM/animal.day)</pre>					
Eu15–28 m	l <sup>(46)</sup>	2 <sup>(12)</sup>	-0.07 [-0.54, 0.39]	0.750	0	0.880
Legume	<b>3</b> (12, 14 and 15)	3 <sup>(22)</sup>	2.08 [1.53, 2.63]	<0.001	0	0.580
Palm	<b>4</b> <sup>(3, 5 and 22)</sup>	I 5 <sup>(82)</sup>	-0.14 [-0.64, 0.36]	0.580	67	<0.001
Crude protein intak	e (g/animal.day)					
Legume	2 <sup>(12 and 14)</sup>	2 <sup>(16)</sup>	314 [93.6, 534]	0.005	81	0.020
Palm	<sup>(4)</sup>	6 <sup>(30)</sup>	-13.8 [-61.5, 33.9]	0.570	0	0.790

Table I. Subgroup analysis for animal performance in tropical grass pastures in silvopastoral systems according to tree types.

<sup>A</sup>Number of studies in each subgroup (superscript numbers refer to list of papers provided in Supplementary file S1).

<sup>B</sup>Number of comparisons in each subgroup (superscript numbers refer to number of animals in each subgroup).

<sup>C</sup>Mean differences between control (pasture monoculture) and silvopastoral systems.

<sup>D</sup>Comparisons were not found in some subgroups and therefore data are not presented.

 $l^2$ , proportion of total variation of size effect estimates due to heterogeneity; Eu I – 14 m, *Eucalyptus* I – 14 m spaced; Eu I 5–28 m, *Eucalyptus* I 5–28 m spaced; Eu 28 m, *Eucalyptus* more than 28 m spaced; Legume, leguminous trees; Palm, palm trees.

## Pasture nutritional value

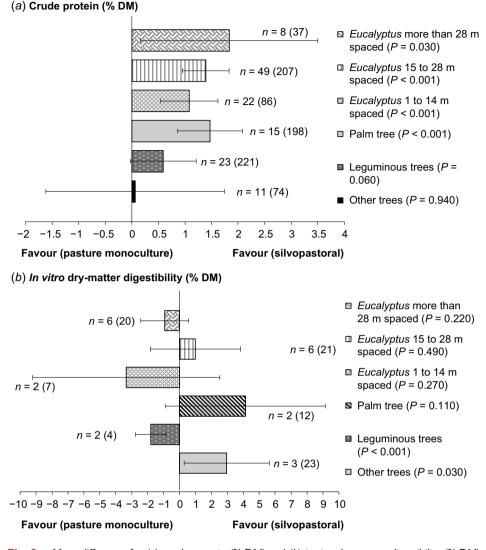
Crude protein content was higher in Eu1–14 m (MD = 1.08; P < 0.001), Eu15–28 m (MD = 1.39; P < 0.001) and Eu28 m (MD = 0.83; P = 0.03) (Fig. 3) than in pasture monoculture. Palm tree SPSs also had higher crude protein content (MD = 1.47; P < 0.001) than did pasture monoculture. SPSs with leguminous trees and with other types of tree had crude protein contents similar (P > 0.05) to those of pasture monoculture. The *in vitro* DM digestibility was lower in SPSs with leguminous trees (MD = -1.81; P < 0.001) and higher in SPSs with other types of tree (MD = 2.96; P = 0.03), than that in pasture monoculture. There was no difference (P > 0.05) between the other SPS subgroups and pasture monoculture.

Dry-matter content was lower in palm trees than in pasture monoculture (P = 0.01), but similar in the other SPS subgroups (P > 0.05; Table 2). Heterogeneity was high in SPSs with palm trees and with other types of tree (P < 0.001). Neutral detergent fibre was lower in Eu15–28 m (MD = -2.36;

P < 0.001) and Eu28 m (MD = -1.12; P = 0.02) than in pasture monoculture, while there was no difference for the other SPS subgroups (Table 2). Heterogeneity was high in Eu1–14 m, Eu15–28 m and leguminous trees (P < 0.01). Acid detergent fibre was higher in SPSs with other types of tree (MD = 1.80; P < 0.001) than in pasture monoculture, while there was no difference for the other SPS subgroups (Table 2). Lignin content was higher in Eu28 m (MD = 1.11; P < 0.001) than in pasture monoculture, while there was no difference for the other SPS subgroups (Table 2). Lignin content was higher in Eu28 m (MD = 1.11; P < 0.001) than in pasture monoculture, while there was no difference for the other SPS subgroups. *In situ* DM digestibility was higher in palm trees (P < 0.001) than in pasture monoculture, while there was no difference for the other SPS subgroups. *In situ* DM digestibility was higher in palm trees (P < 0.001) than in pasture monoculture, while there was no difference for the other SPS subgroups. (Table 2).

## **Pasture agronomic characteristics**

Forage mass was lower in all SPS subgroups than in pasture monoculture (P < 0.01; Fig. 4). Among *eucalyptus* SPSs, the difference from pasture monoculture was greater for Eu1–14 m (MD = -2.24; P < 0.001) than for Eu28 m (MD = -0.50;



**Fig. 3.** Mean difference for (*a*) crude protein (% DM) and (*b*) *in vitro* dry-matter digestibility (% DM) contents of tropical grasses in silvopastoral systems according to tree type. *n* following each bar on the graph indicates the number of comparisons in each subgroup and the number inside parentheses indicates the number of forage samples.

P < 0.001). Forage density was lower in all SPSs than in pasture monoculture (P < 0.001), with greater reductions in Eu1–14 m (MD = -58.4) than in Eu28 m (MD = -18.5).

Pasture height was lower in Eu1–14 m (MD = -7.21; P < 0.001; Table 3) and higher in Eu28 m (MD = 1.78; P = 0.002) than that in pasture monoculture, with no difference for the other SPS subgroups. Heterogeneity was high in Eu1–14 m and Eu15–28 m (P < 0.001). Tiller number was lower in Eu28 m (MD = -207; P < 0.001) and in SPSs with other types of tree (MD = -707; P = 0.03) than that in pasture monoculture, with no difference for the other SPS subgroups (Table 3). Leaf area index was lower in palm tree SPSs than in pasture monoculture (P < 0.001), and with no difference for the other SPS subgroups (Table 3). Specific leaf area was higher in Eu1–14 m (MD = 39.3;

P = 0.05), Eu28 m (MD = 11.4; P = 0.003) and leguminous trees (MD = 25.3; P = 0.03) than in pasture monoculture (Table 3). Heterogeneity was high in Eu1–14 m and Eu15–28 m (P < 0.001).

Plant leaf percentage was higher in Eu28 m (MD = 2.72; P = 0.03) than in pasture monoculture, but with no difference found for the other SPS subgroups (Table 3). Heterogeneity was high in all SPS subgroups (P < 0.01). The plant stem percentage was also higher in Eu28 m (MD = 1.86; P < 0.001) than in pasture monoculture, but this was offset by a lower percentage dead. There was no difference in plant stem percentage for the other SPS subgroups (Table 3). Plant dead material percentage was lower in Eu15–28 m (MD = -2.85; P = 0.03) and Eu28 m (MD = -4.05; P < 0.001) than in pasture monoculture, but with no difference for the monoculture, but with no difference for the monoculture.

Subgroup	Number of studies <sup>A</sup>	N <sup>B</sup>	<b>MD (95% CI)</b> <sup>C</sup>		Heterogeneity	
			Random effect	P-value	l <sup>2</sup> (%)	P-value
Dry matter (% fresh	matter)					
Legume	I (1)	6 <sup>(60)</sup>	-0.79 [-1.65, 0.07]	0.070	0	0.910
Palm	2 <sup>(22 and 53)</sup>	5 <sup>(24)</sup>	-4.45 [-7.81, -1.09]	0.010	84	<0.001
Other trees	6 <sup>(22, 35, 40, 63, 64 and 65)</sup>	I 7 <sup>(85)</sup>	0.71 [-1.58, 3.00]	0.540	93	<0.001
Neutral detergent fit	ore (% dry matter)					
Eul-14 m	<sup>(9)</sup>	4 <sup>(16)</sup>	-0.01 [-1.52, 1.50]	0.990	88	0.001
Eu15–28 m	<b>4</b> (8, 23, 37 and 46)	I 0 <sup>(39)</sup>	-2.36 [-3.51, -1.20]	<0.001	85	<0.001
Eu28 m	5 <sup>(23, 29, 32, 61 and 66)</sup>	2 <sup>(40)</sup>	-1.12 [-2.10, -0.15]	0.020	43	0.080
Legume	3 <sup>(8, 38 and 55)</sup>	7 <sup>(29)</sup>	2.33 [-2.41, 7.06]	0.340	87	<0.001
Palm	<b>4</b> <sup>(3, 5, 22 and 59)</sup>	I 5 <sup>(198)</sup>	-1.15 [-2.86, 0.56]	0.190	28	0.150
Other trees	6 <sup>(9, 13, 17, 40, 63 and 64)</sup>	I I <sup>(58)</sup>	-0.48 [-1.15, 0.20]	0.170	0	0.940
Acid detergent fibre	(% dry matter)					
Eu15–28 m <sup>D</sup>	l <sup>(23)</sup>	3(9)	-0.33 [-1.11, 0.44]	0.400	0	0.570
Eu28 m	<b>4</b> <sup>(23, 29, 32 and 66)</sup>	7 <sup>(32)</sup>	0.49 [–0.92, 1.91]	0.500	89	<0.001
Legume	2 <sup>(8 and 38)</sup>	5 <sup>(13)</sup>	0.22 [-1.03, 1.47]	0.730	0	0.480
Palm	<b>4</b> (3, 5, 22 and 59)	I 5 <sup>(198)</sup>	-0.38 [-1.17, 0.41]	0.340	0	0.800
Other trees	5 <sup>(8, 13, 40, 63 and 64)</sup>	6 <sup>(38)</sup>	1.80 [0.80, 2.80]	<0.001	35	0.170
Lignin (% dry matter	)					
Eu15–28 m	l <sup>(49)</sup>	25 <sup>(50)</sup>	0.05 [-0.13, 0.23]	0.600	99	<0.001
Eu28 m	2 <sup>(29 and 32)</sup>	3 <sup>(20)</sup>	1.11 [0.52, 1.70]	<0.001	23	0.270
Palm	3 <sup>(3, 5 and 59)</sup>	I 3 <sup>(186)</sup>	0.64 [-0.40, 1.68]	0.230	52	0.010
In situ dry-matter dig	estibility (% dry matter)					
Legume	<sup>(8)</sup>	3 <sup>(9)</sup>	-0.47 [-12.3, 11.4]	0.940	0	0.990
Palm	<sup>(5)</sup>	6 <sup>(120)</sup>	2.92 [0.11, 5.72]	0.040	90	<0.001
Other trees	2 <sup>(8 and   3)</sup>	2 <sup>(15)</sup>	-3.03 [-6.61, 0.55]	0.100	0	0.810

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I able 2.	Subgroup anal	vsis for chemica	composition of th	rodical grasses il	n siivodastoral :	systems according to tree type.

<sup>A</sup>Number of studies in each subgroup (superscript numbers refer to list of papers listed in Supplementary file S1).

<sup>B</sup>Number of comparisons in each subgroup (superscript numbers refer to number of forage samples in each subgroup).

<sup>C</sup>Mean differences between control (pasture monoculture) and silvopastoral systems.

<sup>D</sup>Comparisons were not found in some subgroups and therefore data are not presented.

l<sup>2</sup>, proportion of total variation of size effect estimates due to heterogeneity; Legume, leguminous trees; Palm, palm trees; Eu I – I 4 m, Eucalyptus I – I 4 m spaced; Eu I 5 – 28 m, Eucalyptus I 5 – 28 m, Eucalyptus I 5 – 28 m, Spaced; Eu 28 m, Eucalyptus more than 28 m spaced.

other SPS subgroups (Table 3). Heterogeneity was high in Eu15–28 m and Eu28 m (P < 0.001).

## Publication bias risk and methodological quality of the study

The evaluation of publication bias risk by using the funnel plot showed a uniform distribution between standard deviation and mean difference of the comparisons present in the articles (Fig. S2; Supplementary material S1). Descriptions of treatments, experimental design, descriptive statistics of results and experimental procedures were well provided in the articles of SPSs with tropical grasses (Fig. S1; Supplementary material S1). However, descriptions of the specific sample size for each variable, sample size determination, randomisation,

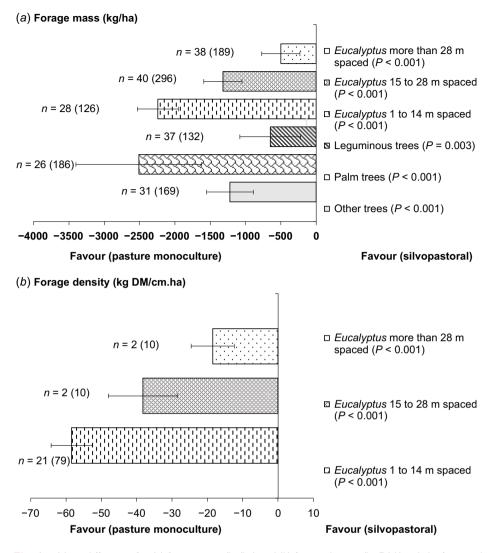
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methods used to verify statistical assumptions, data access, conflict of interest and funding sources were flawed among the selected articles.

## Discussion

### **Study characteristics**

The countries where most of the studies were conducted (Brazil, Mexico and Colombia) normally have high rain volumes concentrated in some months. This fact shows that water may not be an extremely limiting factor for the agricultural systems in these countries in the months with high rainfall. However, in months or countries with less rainfall, competition for water can be a problem within



**Fig. 4.** Mean difference for (*a*) forage mass (kg/ha) and (*b*) forage density (kg DM/cm.ha) of tropical grasses in silvopastoral systems according to tree type. *n* following each bar on the graph indicates the number of comparisons in each subgroup and the number inside parentheses indicates the number of forage samples.

systems with a high tree density. Brazil was the country with the highest number of studies, which shows the importance of this country in the development of integrated systems in tropical regions. Furthermore, SPSs with different *Eucalyptus* arrangements and leguminous trees accounted for 63% of the total number of studies, which indicates the dominance of these systems. *Eucalyptus* use occurs mainly in Brazil due to the standardisation of production processes for the implementation of the arboreal component in the first years of crop–livestock–forest integration (Balbino *et al.* 2011). Other factors that encourage *Eucalyptus* use in SPSs in Brazil are the high quality of the wood, the well established consumer market for the wood, rapid growth, great diversity of species adapted to different climates and soil conditions, low seedling price and ease of cutting (Stape *et al.* 2010). The main forages used in the studies were *U. decumbens*, *U. brizantha* and *M. maximus. Urochloa decumbens* is recognised for its greater resistance to shading (Guenni *et al.* 2008; Gómez *et al.* 2012; Lima *et al.* 2018) and, therefore, has been used in a large number of studies. However, more productive species, such as *U. brizantha* cv. Marandu and cv. Piatã and *M. maximus*, were also used in many systems, probably with the objective of increasing pasture production and the stocking rate of these systems. The results showed reductions in pasture and animal production in SPSs with a higher tree density, which shows the need to develop more productive forage species for these systems.

Most of the included studies conducted only one assessment or short-term (annual) assessment. This is an important negative aspect of the studies because SPSs are long-term

Subgroup	Number of studies <sup>A</sup>	N <sup>B</sup>	<b>MD (95% CI)</b> <sup>C</sup>		Heterogeneity	
			Random effect	P-value	l <sup>2</sup> (%)	P-value
Pasture height (cm)	)					
Eul-I4 m	5 <sup>(9, 48, 50, 52 and 58)</sup>	24(110)	-7.21 [-10.9, -3.56]	<0.001	100	<0.001
Eu15–28 m	6 <sup>(9, 19, 23, 37, 50 and 58)</sup>	I 6 <sup>(70)</sup>	3.10 [-0.73, 6.92]	0.110	100	<0.001
Eu28 m	3(19, 23 and 29)	5 <sup>(24)</sup>	1.78 [0.64, 2.91]	0.002	17	0.300
Legume	2 <sup>(I and 8)</sup>	9 <sup>(69)</sup>	1.48 [-1.37, 4.34]	0.310	0	0.900
Palm	<sup>(59)</sup>	3 <sup>(90)</sup>	0.35 [-2.30, 3.00]	0.800	0	0.940
Other trees	her trees 6(8, 13, 26, 35, 45 and 65)		-2.81 [-13.6, 8.02]	0.610	79	<0.001
Tiller number (tille	rs/ha)					
Eu I–14 m <sup>D</sup> I <sup>(52)</sup>		2 <sup>(4)</sup>	-170 [-381, 41.2]	0.110	0	0.730
Eu28 m	4 <sup>(25, 31, 33 and 42)</sup>	I 3 <sup>(46)</sup>	-207 [-297, -117]	<0.001	87	<0.001
Palm	<b>3</b> (6, 53 and 59)	I2 <sup>(120)</sup>	-52.2 [-171, 66.5]	0.390	97	<0.001
Other trees	2 <sup>(13 and 45)</sup>	4 <sup>(32)</sup>	-707 [-1357, -56.7]	0.030	86	<0.001
Leaf area index						
Eu28 m	4 <sup>(19, 24, 25 and 29)</sup>	8 <sup>(87)</sup>	0.35 [-0.11, 0.80]	0.130	74	0.003
Palm	<sup>(53)</sup>	3 <sup>(12)</sup>	-1.99 [-3.00, -0.97]	<0.001	88	<0.001
Specific leaf area (c	m²/g)					
EuI–I4 m	l <sup>(58)</sup>	2 <sup>(6)</sup>	39.3 [-0.06, 78.7]	0.050	99	<0.001
Eu15–28 m	<sup>(58)</sup>	2 <sup>(6)</sup>	16.6 [-5.26, 38.4]	0.140	96	<0.001
Eu28 m	2 <sup>(24 and 25)</sup>	6 <sup>(72)</sup>	.4 [3.87,  8.9]	0.003	0	0.790
Legume	[ <sup>(1)</sup>	<b>6</b> <sup>(60)</sup>	25.3 [3.05, 47.5]	0.030	0	0.860
Plant leaf percentag	ge (%)					
EuI–I4 m	2 <sup>(50 and 52)</sup>	6 <sup>(36)</sup>	6.12 [-1.44, 13.7]	0.110	74	0.002
Eu15–28 m	<b>3</b> <sup>(2, 23</sup> and 50)	<b>9</b> <sup>(41)</sup>	0.81 [-3.07, 4.68]	0.680	98	<0.001
Eu28 m	7 <sup>(20, 21, 23, 24, 25, 32 and 61)</sup>	19 <sup>(121)</sup>	2.72 [0.27, 5.16]	0.030	93	<0.001
Legume	(62)	16(192)	-1.69 [-6.55, 3.16]	0.490	88	<0.001
Other trees	<b>4</b> <sup>(13, 26, 45 and 65)</sup>	<sup>(72)</sup>	-3.40 [-8.99, 2.19]	0.230	81	<0.001
Plant stem percenta	age (%)					
Eul-14 m	2 <sup>(50 and 52)</sup>	6 <sup>(36)</sup>	1.98 [-6.04, 10.0]	0.630	68	0.009
Eu15–28 m	<b>3</b> (2, 23 and 50)	9 <sup>(41)</sup>	0.50 [-2.49, 3.48]	0.740	93	<0.001
Eu28 m	<b>7</b> <sup>(20, 21, 23, 24, 25, 32 and 61)</sup>	<b>19</b> <sup>(121)</sup>	1.86 [1.03, 2.69]	<0.001	50	0.007
Legume	(62)	I6 <sup>(192)</sup>	2.09 [-3.04, 7.22]	0.420	96	<0.001
Other trees	3 <sup>(13, 26 and 65)</sup>	8(52)	4.01 [-2.44, 10.5]	0.220	89	<0.001
Plant dead material	percentage (%)					
Eul-14 m	<sup>(52)</sup>	2 <sup>(12)</sup>	2.11 [-1.03, 5.25]	0.190	0	0.610
Eu15–28 m			-2.85 [-5.48, -0.21]	0.030	98	<0.001
Eu28 m	<b>7</b> (20, 21, 23, 24, 25, 32 and 61)	5 <sup>(17)</sup> 19 <sup>(121)</sup>	-4.05 [-6.00, -2.09]	<0.001	86	<0.001
Legume	[ <sup>(1)</sup>	6 <sup>(60)</sup>	-0.83 [-1.75, 0.10]	0.080	0	0.830
Other trees	2 <sup>(13 and 26)</sup>	<b>6</b> <sup>(16)</sup>	-1.22 [-5.07, 2.63]	0.530	0	0.480

Table 3. Subgroup analysis for agronomic characteristics of tropical grasses in silvopastoral systems according to tree type.

<sup>A</sup>Number of studies in each subgroup (superscript numbers refer to list of papers listed in Supplementary file SI).

<sup>B</sup>Number of comparisons in each subgroup (superscript numbers refer to number of forage samples in each subgroup).

 $^{\rm C}{\rm Mean}$  differences between control (pasture monoculture) and silvopastoral systems.

<sup>D</sup>Comparisons were not found in some subgroups and therefore data are not presented.

 $l^2$ , proportion of total variation of size effect estimates due to heterogeneity; Eu I – 14 m, *Eucalyptus* I – 14 m spaced; Eu I 5–28 m, *Eucalyptus* I 5–28 m spaced; Eu 28 m, *Eucalyptus* more than 28 m spaced; Legume, leguminous trees; Palm, palm trees.

systems and very dynamic over the years. For example, as *Eucalyptus* growth increases over the years, pasture shading can progressively reduce system production, which demonstrates the need for longer evaluation periods. Additionally, in SPSs that intercrop leguminous shrubs with grass and legume pastures, animal selectivity for forage with higher nutritional value and low resistance to shading can compromise system durability (Alonso Lazo *et al.* 2006; Paciullo *et al.* 2014, 2021).

## Animal performance

There was no influence on average daily gain for any SPS subgroup because the amount of forage offered to animals raised in SPS and pasture monoculture was standardised in the studies included in this meta-analysis. There was lower weight gain per area in Eucalyptus SPSs with less than 28 m spacing between rows and higher in those with more than 28 m. This change in weight gain per area occurred because, although all Eucalyptus SPSs experienced a reduction in biomass production, this reduction was not very great in studies with more than 28 m between rows (6280 vs 6330 kg DM/ha). The lower biomass production in Eucalyptus SPSs with more than 28 m between rows did not reduce stocking rate. Under these conditions, the significant improvement in pasture nutritional value observed in the present study and the probable improvement in animal comfort allowed greater total weight gain per unit area.

These results are important because they indicate that SPSs with more than 28 m between rows may have much higher total system profitability (coming from livestock and forestry) than does pasture monoculture (Magalhães et al. 2018). The results of the present review showed reductions of more than 50% in weight gain per area in SPSs with less than 28 m between rows, which was also found by Oliveira et al. (2014), Santos et al. (2018) and Pereira et al. (2021). These results need to be considered by land managers and policy makers because the implementation of systems with lower animal production may reduce total system profitability and the use of these systems on commercial farms due to lower financial sustainability. It is important to note that to obtain the environmental benefits of a better balance between emissions reduction and biodiversity with agricultural productivity, these systems need to be implemented on a large scale in commercial farms.

There was no change in weight gain per area in SPSs with leguminous and palm trees, compared with pasture monoculture, but it was higher in the SPSs with other types of tree, than in pasture monoculture. These results showed that it is possible to develop productive systems with livestock and forestry activities together, such as cattle breeding and production of babassu (*Attalea speciosa*) or coconut (*Cocos nucifera*) (Pandey *et al.* 2011; Frota *et al.* 2017; Araújo *et al.* 2020), which can also increase total system production compared with systems with only livestock in pasture monoculture. Results that demonstrated similar or superior weight gain per area in SPSs are important because they indicate increased system productivity, which can increase profitability and SPS utilisation on commercial farms in a tropical climate (Gil *et al.* 2015). Furthermore, there are still extensive beef cattle production systems in Brazil that use degraded pastures and the results of the present study suggest that SPSs are capable of improving productivity indicators of these areas (Figueiredo *et al.* 2017).

There were superior DM and crude protein intakes in SPSs with leguminous trees. These systems show greater species diversification and the use of leguminous plants improves the nutritional value of the ingested pasture (Epifanio et al. 2019), which explains the higher protein intake. In addition to better pasture nutritional value, the better climatic conditions (lower temperature and direct radiation) also improve the thermal environment of SPSs, which can improve animal comfort and increase the grazing period during the day. This phenomenon may have generated the highest DM intake (Domiciano et al. 2016; Giro et al. 2019; Oliveira et al. 2019). Few studies have determined DM intake in other SPSs, which makes it necessary to conduct more studies to understand how pasture morphological and nutritional alterations affect cattle intake in non-leguminous SPSs with tropical grasses.

There was no change in milk production in Eucalyptus SPSs with 15–28 m between rows, although only two studies (five comparisons) assessed milk production in this type of system. There was higher milk production in SPSs with leguminous trees, probably due to the better pasture nutritional value and animal comfort. Studying dairy cows grazing on stargrass (Cynodon nlemfuensis) and Leucaena leucocephala trees, Bottini-Luzardo et al. (2016) found that animals had higher metabolisable energy intake in SPSs than in monocultures (161 vs 131 MJ/cow.day), however, they had the same milk production. In addition, animals in SPSs had higher blood urea nitrogen, which may indicate imbalance in ruminal protein metabolism and nitrogen loss. Therefore, special attention should be given to diet adjustment in these SPSs with dairy animals and L. leucocephala, to increase cow efficiency. None of the selected studies evaluated milk production per unit area in SPSs. This assessment is important because, due to the reduction in forage mass and stocking rate, a reduction in total production can occur, which would compromise production system profitability.

## Pasture nutritional value

There was higher crude protein content in SPSs with *eucalyptus* and palm trees than in pasture monoculture. This increase in crude protein is attributed to increased soil nitrogen (N), which occurs due to litter fall from trees and increased N cycling from deeper soil layers because tree roots reach deeper layers than do grass roots (Wilson 1996; Baah-Acheamfour *et al.* 2015; Moreira *et al.* 2022). Another

factor that explains the increase in crude protein is the dilution of N in the plant in pasture monoculture due to higher forage mass (Lemaire and Chartier 1992; Kephart and Buxton 1993). According to the nitrogen dilution theory, plants extract an ideal percentage of soil N for a given level of dry-mass production (Lemaire and Chartier 1992). Thus, if the plant grows under shade, it does not metabolise and convert all of the N absorbed into dry-mass accumulation, which reduces the efficiency of plants in converting absorbed N into biomass production. In addition, the delayed ontogenic development of shaded plants keeps them physiologically younger (Taiz et al. 2015), which may reduce fibre accumulation and might increase crude protein proportionally (Paciullo et al. 2007; Sousa et al. 2010; Santos et al. 2018). This increase in crude protein content is a positive aspect and can improve individual animal performance in SPSs; however, due to the large reduction in forage mass observed in denser systems, crude protein production is also reduced, which can reduce pasture stocking rate and animal productivity in such SPSs.

There was no well defined pattern of variation in *in vitro* DM digestibility among subgroups, with inferiority in SPSs with leguminous trees, superiority in SPSs with other types of tree and equality in the other SPS subgroups. This variable behaviour occurs because there are factors that potentially increase, and others that reduce, *in vitro* DM digestibility of tropical grasses in SPSs. For example, plants in the *Eucalyptus* subgroup with more than 28 m between the rows were taller and had a greater stem proportion, which may increase fibrous fractions and reduce *in vitro* DM digestibility (Santos *et al.* 2018). However, crude protein content was higher and plant dead material percentage was lower in this subgroup, which can proportionally reduce fibrous fractions and increase *in vitro* DM digestibility.

There was lower DM content in SPSs with palm trees than in pasture monoculture, and no change in the other subgroups compared with pasture monoculture. This lower DM content may have generated certain physical limitations to animal intake in this SPS compared with pasture monoculture (Van Soest 1994). Thus, this greater physical limitation may have prevented animals in the SPS from having better DM intake and average daily gain in the present study, even consuming better-quality forage. Other factors, such as pastures with worse structure with lower density and higher proportion of stems in the upper stratum in systems with higher tree density, may also have prevented animals in the SPS from having better DM intake, even consuming better-quality forage (Mezzalira *et al.* 2014; Geremia *et al.* 2018).

Grasses in some SPS subgroups had neutral detergent fibre reductions and acid detergent fibre and lignin increases, but also without any consistent pattern of change among subgroups. This reduction in neutral detergent fibre may be due to changes in plant ontogenic development. Deinum *et al.* (1996) observed that, under low light incidence (2.6 MJ m<sup>-2</sup>), *U. brizantha* and *M. maximus* had fewer

sclerenchyma cells (supporting cells), which also had narrower cell walls (1.5 vs 2.5  $\mu$ m), than did cells subjected to high light intensity (17.4 MJ m<sup>-2</sup>). These changes generate thinner leaves and a lower fibre content (Gómez *et al.* 2012), which explains the neutral detergent fibre reduction in some SPS subgroups. The increase in lignin content was probably due to the increase in stem proportion, which is a plant adaptation to increase height and reach light in pasture upper strata (Santos *et al.* 2016). Our results did not show significant and consistent improvement in fibrous fractions.

## **Pasture agronomic characteristics**

There was lower forage mass in all SPSs. This lower pasture yield also reduced weight gain per area in denser Eucalyptus SPSs. The lower forage mass in SPSs is mainly due to lower light availability and competition for water and nutrients between trees and grasses, which reduces photosynthetic rate and photoassimilate accumulation (Gómez et al. 2012; Guenni et al. 2018). Nascimento et al. (2019) observed that U. brizantha cv. Marandu had a photosynthetic rate 51.9% greater (36.3 vs 23.9  $\mu$ mol CO<sub>2</sub>/m<sup>2</sup>.s) and a transpiration rate 14.0% greater (3.91 vs 3.43 mol H<sub>2</sub>O/m<sup>2</sup>.s) in the centre of rows than in grass immediately below the Eucalyptus trees. These results are important because they indicate the need to use systems with a greater row spacing and lower tree density to reduce light limitation and competition between trees and grasses for water and nutrients, so as to increase production and profitability from livestock. In tropical countries with higher rainfall volumes, this competition for water may not be very significant. However, in countries with less rainfall, competition for water can be a problem in systems with a high tree density, which indicates the need for greater care in planning the system to avoid reduction in pasture and animal productions.

Plants under conditions of moderate shading show morphological changes to compensate for the reduction in luminosity (Paciullo *et al.* 2011), which was demonstrated in the present study by the greater pasture height and specific leaf area to maintain biomass production. At the leaf level, the main alterations of grasses under shading are a smaller volume of support tissue and greater chlorophyll concentration (Lambers *et al.* 2008; Gómez *et al.* 2012, Nascimento *et al.* 2019). However, our results showed that these compensatory mechanisms were not effective in systems with a higher tree density.

Araújo *et al.* (2020) showed that *U. brizantha* cv. Marandu had higher rates of tissue renewal and tiller appearance in monoculture or under moderate shade than under more severe shading. These results indicated lower tiller production for grass under shade, which corroborates our results of lower tiller number, forage density and forage mass. Shading produces wavelength irradiation with a lower proportion of blue and a greater proportion of far-red, which alters phytochrome structure and plant metabolism (Ballaré 1999) and reduces basal tiller activation.

Forage density was lower in all SPSs. This indicator demonstrates the structure and capacity of each pasture stratum to provide forage for the animals. The reduction in forage density is explained by the increase in height and reduced forage mass and tiller number. This alteration to pasture structure can alter the grazing dynamics of animals in SPSs. Geremia *et al.* (2018) showed that animals in pastures subjected to intense shading had a higher bite rate and lower bite mass, which may result in longer grazing times to reach the same intake as for animals in pasture monoculture. In addition, the authors observed that the upper pasture stratum in SPSs has more stem and less leaf material than that in pasture monoculture, which can increase the physical barrier and reduce bite mass (Benvenutti *et al.* 2009; Mezzalira *et al.* 2014).

Pasture height was lower in Eucalyptus SPSs with 1-14 m between rows; however, it was higher in those with greater than 28 m spacing. This difference probably occurred because, in very dense systems, light restriction limits plant growth and reduces pasture height. Since light restriction is lower in systems with more than 28 m between the rows, plants elongate stems to reach higher strata and capture more light (Crestani et al. 2017; Geremia et al. 2018), which was confirmed in the present study by the increase in plant stem percentage in such SPSs. The morphological changes of greater height, fewer tillers and lower forage density and pasture leaf area index observed in the present study indicated that pasture management in SPSs needs to be adjusted. For example, Machado et al. (2020) observed that Urochloa decumbens reached 95% of light interception with 20 cm of height in pasture monoculture and with 40 cm in SPS, which indicates that pasture management in SPS should use higher height and grazing interval.

Another strategy observed to increase the photosynthetic efficiency of plants in SPSs was an increase in specific leaf area. This morphological change in shaded plants allows increases in total leaf area, light capture and photosynthetic rate (Guenni *et al.* 2008; Taiz *et al.* 2015). According to Gómez *et al.* (2012), thinner leaves require less energy to build leaf area, which can compensate for lower light availability. The increase in plant leaf percentage was also higher in *Eucalyptus* SPSs with more than 28 m between the rows, which represents another attempt to increase the volume of plant tissue with photosynthetic capacity and compensate for light restriction (Guenni *et al.* 2018).

There was a lower percentage of plant dead material in *Eucalyptus* SPSs with 15–28 m and with more than 28 m between the rows. This was probably due to the rapid nutrient cycling and the lower photosynthetic rates in plants in SPSs, which reduces/delays the senescence process. This change corroborates the increase in crude protein in some SPS subgroups. Plant senescence can be induced by abiotic stresses such as that from UV-B radiation, extreme temperatures and

intense light (Taiz *et al.* 2015). In regions with tropical climate, the high availability of radiation that reaches a plant in pasture monoculture, especially in summer, can generate stress and trigger a cascade of reactions that lead to plant senescence, which explains the greater plant dead-material percentage in pasture monoculture. Furthermore, Santiago-Hernández *et al.* (2016) observed greater metabolic activity of tropical grass cells in pasture monoculture than in SPSs, expressed by higher net  $CO_2$  assimilation rate and stomatal conductance, which may accelerate the senescence process and help explain the higher percentage of plant dead material in pasture monoculture.

## Heterogeneity and publication bias risk

Most of the forage and animal performance variables showed high heterogeneity, which indicates that there are factors other than just the tree type that influence these variables. Variables that possibly increase heterogeneity are the different grass type, system age, climatic seasons, number of trees per hectare, soil quality, distance from row and tree planting direction evaluated within the same SPS subgroup. An alternative to reduce this heterogeneity would be to evaluate data in more SPS subgroups. However, due to our main objective of evaluating the effect of different arboreal arrangements on tropical grasses, it was decided to evaluate the data in subgroups based only on tree type. Furthermore, it is important to note that in addition to shading, competition between trees and pasture for water and nutrients also reduces pasture production and can increase heterogeneity. However, it is very difficult to isolate these effects.

The studies showed a symmetrical distribution in the funnel plot, which indicates a low risk of publication bias. This result is important because it shows that there is no tendency to publish only studies that show the same trend in their results, which gives greater reliability to the results presented in this review (Higgins *et al.* 2019).

## Conclusions

Variations in the availability of light, water, and nutrients in silvopastoral systems improve the nutritional value of tropical grasses due to a consistent increase in crude protein content. There was no pattern of improvement in fibrous fractions and digestibility of tropical grasses in silvopastoral systems. Grasses showed agronomic alterations due to shading and tree/grass competition for moisture or nutrients in all systems. In *Eucalyptus* systems with up to 28 m between rows, silvopastoral systems significantly reduced biomass production and total animal weight gain per area. In those systems with more than 28 m between rows, total animal weight gain area was higher than in pasture monoculture, which indicates the need to use more than 28 m between rows in systems where the main objective is animal

production. Total animal weight gain in systems with leguminous or palm trees was similar to that of pasture monoculture, while for systems with other types of tree, total animal weight gain was higher. These results of equality or superiority in total animal weight gain per area indicate an increase in total system production, which may facilitate the implementation of these silvopastoral systems in commercial farms.

## **Supplementary material**

Supplementary material is available online.

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## 5. CONSIDERAÇÕES FINAIS

A produção de leite e a emissão de metano por vacas mestiças leiteiras é igual em sistemas integrados na região do Cerrado Brasileiro. Os resultados mostraram a necessidade de mais estudos sobre o metabolismo proteico nesses sistemas para entender porque animais consumindo um pasto de melhor valor nutricional não produziram mais leite.

O planejamento de sistemas silvipastoris precisa considerar as condições do mercado local para definir os arranjos arbóreos para maximizar o retorno financeiro e aumentar a utilização desses sistemas em fazendas comerciais no Brasil.

Em locais onde se deseja privilegiar a produção animal deve-se utilizar até 100 árvores por/ha, espaçamento entre renques maior que 28 m e plantio no sentido leste-oeste.