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Can Intensified Pasture Systems Reduce Enteric Methane Emissions from Beef Cattle in the Atlantic Forest Biome?

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Abstract: The objective of this study was to evaluate the effect of different pasture systems on beef steers' performance, dry matter intake, enteric methane emission, carcass production, forage quality, and animal production per hectare (ha). The trial was conducted at Embrapa Southeast Livestock, São Carlos/SP, Brazil. Sixty Cachim beef steers (5/8 Charolais × 3/8 Zebu) with initial liveweights of 255 ± 7 kg were evaluated for two years under five different grazing production systems (EXT = Extensive; INT = Intensive; iCL = Integrated crop livestock; iCLF = Integrated crop livestock forest; iLF = Integrated livestock forest). The final liveweight was greater ($p < 0.05$) for the animals under the INT and iCL systems (484 ± 51 and 466 ± 79 kg, respectively) compared to animals in the iCLF, iLF and EXT systems (416 ± 57, 414 ± 50 and 429 ± 48 kg). The dry matter intake was significantly greater under the EXT system than it was under the iCL system (9.8 ± 2.1 and 7.5 ± 2.9 kg day^{-1}). Regarding the emission intensity in relation to the liveweight gain per unit area (g CH_4 kg LWG^{-1} ha^{-1} year^{-1}), it differed significantly among the systems (EXT = 1.6; INT = 0.6; iCL = 0.8; ICLF = 1.1; ILF = 0.7). Similarly, the methane emission intensity differed in relation to the carcass production (kg CH_4 kg^{-1} carcass; EXT = 0.496; INT = 0.250; iCL = 0.297; iCLF = 0.345; iLF = 0.286). Beef cattle that are raised in intensive and/or integrated pasture systems have a greater availability of forage mass and nutrients than those that are raised extensively. Pasture systems that undergo soil pH correction and fertilization, rotational grazing and/or integrated with maize cropping produce animals with greater average daily gain and final liveweights, thereby lessening the enteric methane emissions per kg of weight gain. In these systems, the efficiency in terms of the gain per land area is also greater, however, the systems that are integrated with a forest component (iLF and iCLF) are equal to that of the EXT system. The same pattern is observed in the intensity of the methane emission as for the efficiency of the animal gain per unit of land area.

Keywords: beef steers; greenhouse gases; integrated crop livestock forest; pasture; Canchim

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1. Introduction

Since the 2000s, the discussion about the contribution of the agricultural sector to global warming, based on greenhouse gas (GHG) emissions, has been increasing year after year. In addition, it is expected that the world's population will reach 9.7 billion people in 2050, which is 36% more than the current figure [1]. Therefore, agricultural activities must grow in line with the growth of the population and the preservation of the environment to sustainably meet this growing demand for food [1].

Globally, grasslands are the dominant form of agriculture by land area, and these are used mainly for the supply of food to ruminants [2,3]. These animals are responsible for two-thirds (2.1 Gt CO_2eq) of the GHG emissions within the agricultural sector due to the methane (CH_4) that is produced during their digestive process [4]. These potent GHGs cannot be overlooked, however, when CH_4 comes from the enteric fermentation of animals that are raised on pastures, it is necessary to consider that this gas within 12 years will be converted into carbon dioxide (CO_2) in the atmosphere. It can thus be captured by the grasses through photosynthesis, ingested by the ruminants and eructed again, making these animals a fundamental component of this nutrient cycle [5].

Pasture systems, when they are well managed, can present significantly lower balances between the emissions and the removals when one is accounting for the atmospheric CO_2 that is absorbed by the grasses, thus promoting carbon stock (C) in the soil [6]. This characteristic of pasture systems reinforces the need to use techniques that can maximize the production from existing pasture areas for the benefit of the environment and livestock production.

The Brazilian Corporation for Agricultural Research (EMBRAPA), in recent years, has been putting efforts into developing, testing, and disseminating pasture systems, seeking to help to intensify livestock production, and at the same time, contribute to greater environmental, social, and economic sustainability [7].

Examples are integrated production systems such as integrated crop–livestock (iCL) systems, integrated crop–livestock–forest (iCLF) systems and integrated livestock–forest (iLF) systems [7,8]. These systems have the potential to recover previously unproductive areas in tropical and subtropical regions, combining agricultural, livestock and forestry production in the same area for a defined period. Adopting these systems can recover the degraded areas at a relatively low cost, producing three times the volume of grain and twice the volume of meat without any need to open new grassland areas [9].

The integrated systems have higher crop yields and improved sustainability relative to the non-grazed areas due to the increased C sequestration, improved nutrient use efficiency and the maintenance of soil quality [10]. Management, intercropping and/or rotation are the basis of these systems and assist in cycling nutrients between plants and animals, improving the production efficiency and maintaining the soil fertility over the long term. The economic benefits include the lower costs of implementation and maintenance when they are compared to conventional systems, with greater profitability, safety, and product quality [11]. All of these characteristics contribute to a reduction in the GHG intensity (emissions per kilogram) of the products.

Understanding how the intensification of pasture farming systems and their integration with cropping and forestry can contribute to greater animal production, while also reducing the GHG emissions from agriculture is needed for the development of policies that encourage the adoption of these systems, and consequently, contribute to environmental preservation [12]. This requires the measurement of the productivity indices and the GHG emissions from these systems, and specifically for enteric methane from grazing livestock [13].

The present work sought to investigate the hypothesis that the intensification of pasture systems can increase the animal performance and animal production per area, by improving the productive and nutritional characteristics of the pasture, and thus, mitigating the emission of enteric methane. Therefore, this study aimed to evaluate the livestock productivity per unit land area, alongside the enteric methane emissions of beef steers that were reared under different pasture systems, including those that were integrated with cropping and forestry practices.

2. Materials and Methods

The experiment was carried out at Embrapa Southeast Livestock, which is located in São Carlos, state of São Paulo, Brazil, at 21°57′33.32″ S 47°50′33.28″ W, at an altitude of 856 m. The climate is subtropical with dry winters and hot, humid summers (Cw type in the Koeppen classification). The experiment consisted of two experimental periods: (a) Period 1: from December 2013 to December 2014; (b) Period 2: from December 2014 to December 2015. This study was approved by the local Committee of Ethics in Animal Experimentation–CEUA (Embrapa–Southeast Livestock) and it is registered with protocol numbers: 02/2011, 07/2011, and 05/2016.

2.1. Systems Establishment and Management

In the iCLF, iCL, iLF and intensive (INT) systems, the soil pH correction process was carried out in 2010 through surface liming and soil nutrient correction based on a soil analysis. After the soil management, the Piatã palisadegrass (*Urochloa* (syn. *Brachiaria*) *brizantha* (Hochst ex A. Rich.) Stapf cv. BRS Piatã) was sowed in a 0.4 m spacing at a rate of 10 kg of seeds ha^{-1}. Limestone was applied annually to increase the base saturation to 60%, fertilizer P (18% P_2O_5) was applied to increase the soil P to 12 mg dm$^{3-1}$ and fertilizer K (KCl, 60% K_2O) was applied to increase the exchangeable K to 3% of the cationic exchange capacity of the soil. The N fertilizer was applied during the rainy season (October–March): (a) Period 1: 157 kg of N ha^{-1}, which was divided into four applications (two as urea—45% N; two as ammonium sulphate—20% N), and (b) Period 2: 202 kg N ha^{-1} applied as urea, which was divided into five applications. In these systems, each repetition area was divided into 6 paddocks, and rotational grazing management was adopted. The steers entered the paddocks when the forage was 35.0 cm high, and they left when it reached 20.0 cm. The extensive system (EXT) was established more than 20 years previously with Urochloa (syn. *Brachiaria decumbens*), but it had not received any interventions prior to the study, and it followed the continuous stocking management process.

The planting of the Eucalyptus seedlings (*Eucalyptus urograndis* clone GG100) in the iCLF and iLF systems took place in April 2011, with trees that were arranged at 2 m spacing in rows, and there was 15 m between the rows (333 trees ha^{-1}) in an east–west direction. In both study periods, the trees were 17.5 m in height and 16.9 cm in diameter on average, providing shading to approximately 50% of the area. Between the tree lines, the solar radiation interception was 45% in the first year, and it was 55% in the second year. In the integrated livestock systems (iCL and iCLF), one-third of the pasture area (two paddocks per repetition area) was renewed each year for the reseeding of the Piatã palisadegrass along with the maize cropping (var. DKR 390 PRO 2), which was harvested for silage. The recommendations of soil correction and fertilization for the maize was based on a soil analysis, following the method of Raij et al. [14]. In both the experimental periods, the maize was sown in November and harvested in March. Figure 1 shows the experimental area which was divided into the respective treatments with a repetition area, and this is characterized below in Table 1.

Table 1. Characterization of the pasture systems by repetition area.

	Pasture System *				
	iCLF	**iCL**	**iLF**	**EXT**	**INT**
Pasture area:	2.7 ha	3.2 ha	2.7 ha	2.9 ha	2.9 ha
Number of paddocks:	6	6	6	1	6
Pasture area during the maize cropping:	1.8 ha	2.1 ha	-	-	-
Grazing method:	Rotational	Rotational	Rotational	Continuous	Rotational
Stocking rate:	Variable	Variable	Variable	Variable	Variable
Soil management:	Yes	Yes	Yes	No	Yes
Integrated crops:	Eucalyptus + Maize	Maize	Eucalyptus	-	-

* EXT: extensive; INT: intensive system; iCL: integrated crop–livestock system; iCLF: integrated crop–livestock–forest system; iLF: integrated livestock–forest system.

Figure 1. Experimental area divided into the respective treatments: (1–2) iCLF: integrated crop–livestock–forest system; (3–4) iLF: integrated livestock–forest system; (5–6) iCL: integrated crop–livestock system; (7–8) EXT: extensive; (9–10) INT: intensive.

2.2. Animals Management

Sixty Canchim steers (30 per period) that were 15 months old with initial live weights (ILW) of 240 ± 23 kg in period one and 285 ± 33 in period two were used in the study. At the beginning of each period, the animals were distributed among five different pasture production systems, with two repetitions (3 animals per area), that corresponded to the treatments: EXT, INT, iCL, iCLF, and iLF.

Throughout the two periods, the animals had ad libitum access to a mineral supplement, and in autumn and winter, they had ad libitum access to a proteinaceous mineral supplement (Table 2).

Table 2. Mineral supplement and proteinaceous mineral supplement.

Spring–Summer		**Autumn–Winter**	
Mineral	**Content (%)** [1]	**Mineral**	**Content (%)** [1]
Phosphor	13.0	Phosphor	1.2
Calcium	21.4	Calcium	8.0
Magnesium	2.3	Magnesium	0.2
Sulfur	4.0	Sulfur	1.5
Zinc	1.0	Zinc	0.064
Copper	0.35	Copper	0.017
Manganese	0.17	Manganese	0.013
Cobalt	0.03	Cobalt	0.001
Iodine	0.03	Iodine	0.012
Fluorine	0.003	Fluorine	0.02
Selenium	0.13	Selenium	0.0003
-	-	Crude protein	40.0

[1] Minimum level per kg of supplement.

In each pasture system, the core animals were maintained throughout the experimental period for assessments of their performance, intake and CH_4 emission. Animals that were external to the experiment ("regulators") were used to adjust the stocking rate according to the forage availability using a "put-and-take" method [15] and visual assessments of the forage availability.

At the beginning and end of each experimental period, the animals were weighed following 16-h fasting period (solids and liquids). The intermediate weighing was conducted every 30 days without fasting. The liveweight gain per hectare (LWG kg ha^{-1}) was calculated by multiplying the number of animals ha^{-1} by the average daily gain (ADG) of the core animals and the total number of grazing days. The ADG was calculated as the difference between the final and initial weights, divided by the number of days in each grazing cycle. In the systems in which rotational management was adopted (INT, iCL, iCLF, iLF), the grazing period lasted for five days, and this was followed by 25 regrowth days, thus totalling 30 days per grazing cycle. In the iCLF and iCL systems, during the maize cultivation, to compensate for the reduction in the pasture area, the four remaining paddocks were grazed for nine days, and this was followed by 27 regrowth days.

The number of animals per area and individual weight were monitored to calculate the stocking rate (AU ha^{-1}), animal performance (kg $animal^{-1}$ day^{-1}) and animal productivity (kg ha^{-1}). The stocking rate expressed in AU ha^{-1} was calculated by adding the average LWG of the core animals with the average LWG of each regulator animal that participated in the adjustment of the stocking rate and multiplying this by the number of animals and days they stayed in each pasture and dividing this by the total number of grazing days in each grazing period.

2.3. Slaughter and Carcass Yield

At the end of each period, the beef steers were slaughtered at an average of 442 ± 62 kg of the liveweight in a commercial slaughterhouse, according to Brazilian RIISPOA guidelines [16]. The carcasses were weighed before being stored in a cold chamber for 24 h at 2 °C to obtain the hot carcass weight (HCW). The carcass yield (%) was calculated as the ratio between the HCW and the SBW.

2.4. Forage Production and Quality

The determination of forage mass and quality was carried out as described by Pezzopane et al. [17]. For each grazing cycle, the accumulation of forage in the pasture systems under rotational stocking (INT, iCL, iCLF and iLF) was calculated by subtracting the forage mass immediately post-grazing (beginning of the rest period) from the forage mass immediately pre-grazing (end of rest period). In the iCL and INT systems, the forage mass was determined by taking representative samples randomly from four locations, following an "N" pattern within a pre-determined paddock. For this, a quadrat metal frame (0.5 × 0.5 m) was placed at each sampling point, and inside the frame, the height of three plants were measured with a millimetered ruler (1 m) from ground level to the curvature of the most recently expanded leaf. Then, the grass was cut to 15 cm above the ground, and the material was weighed. Lastly, the four samples were mixed as a single mixed sample per repetition area. In the iCLF and iLF systems, the same method was carried out in four positions in relation to the eucalyptus lines from the northern lines: 1.5 m (P1); 3.75 m (P2); 7.5 m (P3); 11.25 m (P4). From each mixed sample, a sub-sample was taken to determine the dry matter (DM) content by drying them in an oven (65 °C) for 72 h, and another one was taken to determine the morphological composition (leaf, stem, and dead material). Data on DM content and morphological composition were used to calculate the forage mass (kg DM ha^{-1}).

In the EXT system, the forage mass was evaluated every 12 days using four exclusion cages (0.5 × 0.5 m) which were distributed in an "N" pattern. In this case, the forage accumulation was calculated by subtracting the forage mass when the exclusion cage was installed from the forage mass at the end of the exclusion period (12 days) and adding them to the accumulation of the three periods. The height of the plant was measured at three points inside the metal frame or in the cage with a millimetered ruler (1 m) from the ground level to the top of the most recently expanded leaf. All of the morphological components were weighed separately, and the DM content was determined by drying them in an oven (65 °C) for 72 h.

The samples that were used to determine the DM content were also used to determine the crude protein (CP), neutral detergent fibre (NDF), non-fibrous carbohydrates (NFC) and in vitro digestibility of the matter dry (IVDMD). After drying them, the samples were ground in a Wiley mill with a 0.5 mm screen and analysed using FT-NIR (NIRFlex N500, Buchi, Flawil, Switzerland) using a polarization interferometer. These measurements were performed using a calibration model, which was developed and validated by Embrapa Southeast Livestock specifically for species and cultivars of *Urochloa* spp. For the nutrient production calculations, the nutrient content (%) was multiplied by the forage mass (kg DM ha^{-1}) and divided by 100.

2.5. Pasture Intake

The pasture intake measurements were carried out at the end of each climatic season over the two years of the experiment using titanium dioxide (TiO_2) as an external marker, which was following the methodology of Oliveira et al. [18]. For 12 consecutive days, all of core animals received 20 g of TiO_2 orally, which was divided into two daily supplies and administered rolled up in Kraft paper. On the last five days of dosing, individual faeces samples were collected directly from the rectum of the animals twice a day, which was followed by the TiO_2 dosing.

The interval between the successive dosing and collections was 8 h long, which was a period within the interval that was proposed (8–16 h) by Carvalho et al. [19]. Following the collection, the faeces samples were frozen at −20 °C. At the end of the 5-day collection period, they were defrosted, and the samples were mixed to obtain a single sample per animal. These were dried in an oven at 65 °C for 96 h, and then, ground through a 2 mm sieve. The faeces concentration of the TiO_2 was conducted according to Myers et al. [20].

The indigestible proportion of feed was determined using the internal marker NDFi. The faeces samples that were collected at the same time as the dosing were weighed and dried at 105 °C for 24 h and weighed again after cooling them to room temperature. The samples were ground to 2 mm, then, packed in bags of nonwoven fabric 100 g m^{2-1} [21] and incubated for 240 h in the rumen of the cannulated steers. Following their incubation, the bags were washed under running water until they were completely clear and dried in an oven at 105 °C for 12 h. The concentration of insoluble fibre was determined in a neutral detergent, according to the methodology by Van Soest et al. [22], with the residue in the bags being considered as the NDF content.

The daily total faeces production (TFP) was calculated according to the following equation:

$$\text{TFP (kg day}^{-1}\text{) = Amount of TiO}_2\text{ administered (g)/Concentration of TiO}_2\text{ in faeces (g kg}^{-1}\text{)} \quad (1)$$

The samples for determining the concentration of NDFi of the forage were collected following the methodology proposed by Cook [23], and the determination was conducted following the same methodology as that which was used for the faecal samples. The indigestibility of the forage was calculated by dividing the concentration of NDFi in the forage by the concentration of NDFi in the faeces. Finally, the DMI was calculated as:

$$\text{DMI (kg day}^{-1}\text{) = TFP/Indigestibility of forage} \quad (2)$$

2.6. Enteric Methane Emissions

The enteric methane emissions were measured using the sulphur hexafluoride (SF_6) tracer gas technique [24–26]. Each of the 30 animals was evaluated daily for 24 h for five consecutive days in May, August, November, and February, which are the central months of the four seasons of each year. Permeation tubes with a known SF_6 release rate (1431 ± 59 ng min^{-1}) were administered orally to each of the 30 animals only once, 7 days before the first sampling period. In order to allow the stabilization of the tracer gas flow in the rumen and acclimatize the animals to the equipment, the animals were equipped with halters and adaptation yokes (without the gas collection system) seven days before the CH_4 collection.

At the beginning of each sampling week, the animals were equipped with halters (with a gas collection system attached) with an evacuated PVC U-yoke providing negative pressure to sample the eructated and expired gases, which was configured such that the U-yoke would fill by approximately 50% over a 24 h period. The daily collections began at 7:30 a.m., and the animals were brought from their pasture areas to the handling centre to change the U-yokes every 24 h. The pressure of the U-yokes was measured immediately after the sampling procedure to check the quality of the collection. A lower final pressure than that which was expected could indicate a blocked or disconnected halter, and a higher one than the expected final pressure could indicate a leak.

For the determination of the gas concentrations, the U-yokes were pressurized with N_2 (ultra-pure) to approximately 10% above the atmospheric pressure, and the measurement of CH_4 and SF_6 concentrations was performed using a Shimadzu chromatograph model GC-2014 (Shimadzu Corporation, Kyoto, Japan) following the method described by Johnson et al. [27]. Calibration curves were established using standard gases with a concentration range of 5–20 ppm and 30–1000 ppt for CH_4 and SF_6, respectively. The ambient air samples were also taken at each sampling period for the determination of the ambient CH_4 and SF_6 concentrations.

The CH_4 emissions were calculated using the CH_4:SF_6 ratio in the sampling canister (U-yoke), with each of the gases being corrected for background concentrations, together with the predetermined permeation rate of the SF_6 permeation tubes. The ruminal CH_4 emission rate (RCH_4, g d^{-1}) was determined as:

$$\begin{gathered} RCH_4 = (RSF_6 \times (((CH_4)_M - (CH_4)_{BG})/((SF_6)_M - (SF_6)_{BG})) \times \\ (MWCH_4/MWSF_6)) \times 1000 \end{gathered} \tag{3}$$

where RSF_6 is the release rate of the SF_6 permeation tube (mg d^{-1}); $(CH_4)M$ and $(CH_4)BG$ are the canister and background CH_4 concentrations (ppm), respectively, and $(SF_6)M$ and $(SF_6)BG$ are the respective SF_6 concentrations (ppt), while $MWCH_4$ is the molecular weight of CH_4 (16), and $MWSF_6$ is the molecular weight of SF_6 (146).

Different metrics were established for the CH_4 emission potential, including the g CH_4 per animal per day, kg CH_4 per kg DMI, kg CH_4 per kg ADG, g CH_4 per kg LW and kg CH_4 per kg of the carcass equivalent. This latter one was calculated based on the LWG ha^{-1} $year^{-1}$, which was multiplied by the carcass yield and divided by the CH_4 emission in kg $year^{-1}$.

An area-based metric was also derived to show the intensity of the CH_4 emission in relation to the efficiency of the LWG per area by dividing the methane emission per kilogram of average daily gain (g CH_4 kg ADG^{-1}) by the LWG per hectare per year (LWG ha^{-1} $year^{-1}$).

2.7. Statistical Analysis

The statistical analyses were performed using SAS 9.4 (SAS Inst, Inc., Cary, NC, USA), after verifying the normality of the residues by the Shapiro–Wilk test (PROC UNIVARIATE). The data were analysed following the procedure for mixed models (PROC MIXED), and the seasons were considered as variables which repeated in time (split-plot in time), except for the data after the slaughter. Among the 15 covariance structures that were tested, the chosen one for each variable was based on the lowest value of the corrected Akaike information criteria (AICC) [28].

The model included the treatment effects (five different systems), season (spring, summer, autumn, and winter), period (1 and 2) and the interaction between the treatments and the seasons as fixed effects. The blocks and seasons were considered to be random effects. The ILW was used as a covariate for the final analysis of the DMI, feed conversion (FC), FLW, ADG and for CH_4 emission variables (g day^{-1}, kg kgDMI^{-1}, kg kgADG^{-1}, kgCH_4 kg of eq. carcass^{-1}, gCH_4 kg ADG^{-1}, LWG^{-1} ha^{-1} year^{-1}, respectively). The effects were considered to be significant when $p < 0.05$. All of the means are presented as means corrected by the least-squares methodology, and the means were separated by the Tukey test.

3. Results

3.1. Forage Mass, In Vitro Digestibility and Nutrient Availability

An interaction between the system and the season was observed for all of the variables for the forage nutritive values and availability. Overall, the forage mass (kg DM ha^{-1}) was higher in the INT, iCL and iCLF systems in the spring, summer, and winter, while it was intermediate in the systems with trees (iCLF and iLF) in spring, summer and autumn, and the value of this was the lowest for EXT in summer and autumn. In winter, the tree systems were similar to the EXT one (Table 3; Figure A1).

Table 3. Forage mass, in vitro digestible dry matter (IVDDM), crude protein (CP), neutral detergent fibre (NDF) and non-fibrous carbohydrate availability for the different pasture production systems (two-year averages).

Fixed Effects		**Variables**				
System *	**Season**	**Forage Mass**	**IVDDM**	**CP**	**NDF**	**NFC**
				kg ha^{-1}		
				Main Effects		
EXT		465.90	279.0	39.7	348.1	42.7
INT		1372.07	756.0	120.6	980.1	147.0
iCL		1393.83	755.7	117.6	978.1	170.5
iCLF		924.17	536.7	108.5	619.1	104.2
iLF		686.33	386.1	76.4	469.0	75.4
	Spring	961.09	560.9	100.5	660.3	103.5
	Summer	1291.49	764.7	130.6	890.4	151.7
	Autumn	1102.20	593.5	104.6	774.1	122.7
	Winter	519.06	251.7	34.5	390.7	54.0
				Means		
Mean		968.5	542.7	92.6	678.9	108.0
SEM		61.5	35.3	6.3	42.9	7.8
				Statistical Probabilities		
System		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Season		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
System × Season		0.0004	<0.0001	<0.0001	0.0007	<0.0001

n = 16 for treatment and season; IVDDM In vitro digestibility of dry matter; CP: crude protein; NDF: fibre in neutral detergent; NFC: non-fibrous carbohydrates; * EXT: extensive; INT: intensive system; iCL: integrated crop–livestock system; iCLF: integrated crop–livestock–forest system; iLF: integrated livestock–forest system.

During the spring season, the INT, iCL and iCLF systems had the most digestible forage mass, which were followed by the iLF system together with the EXT one. The systems with the highest production of digestible forage mass in the summer were the INT and iCL systems, which were followed by the tree systems (iCLF and iLF), while the EXT system had the least. In the autumn, the INT system and the iCL system again produced higher amounts of digestible forage mass, which were followed by the iCLF one. The iLF and EXT systems produced the lowest amounts of digestible forage mass during the autumn. Finally, in winter, the greatest availability of digestible forage mass (kg ha^{-1}) was in the INT system, with the iCLF, iLF and EXT systems all being lower, but they did not significantly differ from each other. The iCL system in that same season produced 361 kg ha^{-1} of digestible forage mass, and this was not different from those of the INT, iCLF, iLF and EXT systems (Table 3; Figure A2).

The CP in the forage in the spring season was highest in the iCLF one, intermediate in the INT and iCL ones and lowest in the iLF and EXT ones. In the summer and autumn seasons, the INT and iCL systems produced the largest amounts of CP, which were followed by the iLF system, while the EXT system produced the least. In the winter, the INT and iCL systems produced most CP, which were followed by iCLF, iLF and EXT systems, which did not differ significantly from each other (Table 3; Figure A3).

In spring, the EXT system produced the lowest amount (kg ha^{-1}) of available NDF when it was compared to the other systems. In summer, the INT and iCLF systems were superior to iCL and iLF ones, which in turn were superior to the EXT one. In the autumn season, the INT system had an NDF higher than the iCL, iCLF and iLF ones did. The extensive system during this same season had lower NDF values than the others did. In winter, the INT and iCLF systems had a greater availability of NDF (kg ha^{-1}) when they were compared to that of the others (Table 3; Figure A4).

Regarding the content of non-fibrous carbohydrates, the INT, iCL and iCLF systems during the spring season showed a greater availability (kg ha^{-1}) when they were compared to the iLF system, which in turn was superior to the EXT system. In the summer, the iCL system was superior to all of the others; in order, the INT system was inferior to the iCL, but it was superior to the iCLF, iLF and EXT systems. The systems with trees did not differ but were still superior to the EXT system. In the autumn season, the INT and iCL systems did not differ from each other, and they were superior to the others. The iCLF system was superior to the iLF one, which in turn, was superior to the EXT system. Finally, in the winter, the INT and iCL systems, as in the autumn, did not differ and were superior to the other systems, which also did not differ between the same types (Table 3; Figure A5).

3.2. Animal Performance and Methane Emissions

The ILW of the steers did not show any significant difference (Table 4), whereas the FLW was higher ($p < 0.05$) for the animals of the INT and iCL systems compared to those of the iCLF, iLF and EXT systems (Table 4). There were significant differences in the DMI ($p < 0.05$) between the animals of the iCLF system and the EXT one, with the former being greater than EXT one, while animals from the INT, iCL and iLF systems were not significantly different to the iCLF or EXT ones (Table 4).

Table 4. Performance variables and CH_4 emission of cattle raised in different pasture production systems in Brazil southeast (means of two years).

Variables	**Systems ***							
	n	**EXT**	**INT**	**iCL**	**iCLF**	**iLF**	**SEM**	***p*-Value**
ILW (kg)	60	253	267	256	267	267	8.39	0.5940
FLW (kg)	60	429 ^{b}	484 ^{a}	466 ^{a}	416 ^{b}	414 ^{b}	16.76	<0.0001
DMI (kg day^{-1})	60	9.8 ^{a}	8.7 ^{ab}	7.5 ^{b}	8.9 ^{ab}	8.3 ^{ab}	0.31	<0.0001
LWG (kg ha^{-1} year^{-1})	60	290 ^{c}	615 ^{a}	487 ^{ab}	385 ^{bc}	497 ^{ab}	53.98	<0.0001
CH_4 (g day^{-1})	60	199.7	226.1	209.8	180.9	196.7	7.3	0.1606
CH_4 (g kg LW^{-1})	60	0.62	0.58	0.61	0.53	0.58	0.03	0.2047
CH_4 (kg kgDMI^{-1})	60	0.028 ^{a}	0.028 ^{a}	0.029 ^{a}	0.022 ^{b}	0.025 ^{ab}	0.001	<0.0001
gCH_4 kgADG^{-1} LWG ha^{-1} year^{-1}	60	1.6 ^{a}	0.6 ^{c}	0.8 ^{bc}	1.1 ^{ab}	0.7 ^{bc}	0.09	0.0031
kgCH_4 kg Carcass eq.$^{-1}$	60	0.496 ^{a}	0.250 ^{b}	0.297 ^{b}	0.345 ^{b}	0.286 ^{b}	0.024	0.0047

^{a,b,c} Means with different letters on the same line differ statistically at $p \leq 0.05$. LW = liveweight; SEM = standard error of means; * EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

When we were evaluating the LWG ha^{-1} $year^{-1}$, the EXT system was the one with the lowest animal production ($p < 0.05$) when it was compared to the INT system. The iCL and iLF systems did not differ from each other and showed LW productions per hectare that were similar to the INT and iCLF systems, but higher than the EXT one. Finally, the LWG ha^{-1} $year^{-1}$ in the iCLF system was lower than it was the INT system, but it was similar to the EXT system (Table 4).

The CH_4 emission, expressed in grams per day and in grams per kilogram of LW, did not show any significant difference between the treatments. However, there were significant differences ($p < 0.05$) in the emissions in relation to the dry matter intake (kg CH_4 kg DMI^{-1}), with the EXT, INT and iCL systems having higher emissions when they were compared to the iCLF system, whereas the iLF system was not significantly different to any of the other systems (Table 4). The emission intensity, expressed as g CH_4 kg ADG^{-1} LWG ha^{-1} $year^{-1}$ area, was significantly lower for the INT system when it was compared to the EXT and iCLF systems, but it did not differ from the iCL and iLF systems. The CH_4 emission intensity (Table 4) which was calculated in relation to carcass production (kg CH_4 kg of $carcass^{-1}$) was greatest in the EXT system when it was compared to all of the other systems, which did not differ significantly.

There were significant interactions between the systems and the seasons in animal the stocking rate, and in spring and winter, all of the systems were similar, while in summer and autumn, the stocking rate was significantly lower for EXT one (Table 5; Figure A6).

Table 5. Animal stocking rate, feed conversion, average daily gain and CH_4 emissions per kg of ADG of cattle raised in different pasture production systems in Brazil southeast (means of two years).

Fixed Effects		**Variables**			
Systems *	**Season**	**Stocking Rate ($AU\ ha^{-1}$)**	**FC ($kgDM\ kgLW^{-1}$)**	**ADG ($kg\ day^{-1}$)**	**CH_4 ($kg\ kgADG^{-1}$)**
EXT		1.3	26.2	0.46	0.465
INT		2.3	17.0	0.59	0.378
iCL		1.7	14.9	0.58	0.343
iCLF		1.8	20.6	0.43	0.441
iLF		2.1	22.5	0.44	0.450
	Spring	1.3	12.7	0.67	0.342
	Summer	2.5	11.9	0.68	0.293
	Autumn	2.7	24.9	0.42	0.562
	Winter	0.96	31.5	0.23	0.466
			Means		
Mean		1.8	20.2	0.50	0.416
SEM		0.10	3.3	0.02	0.053
			p values		
System		<0.0001	<0.0001	<0.0001	0.0031
Season		<0.0001	<0.0001	<0.0001	<0.0001
System × Season		0.0049	0.0201	<0.0001	0.0007

n = 60 for treatment and season/AU: animal unit; ADG: average daily gain; FC: feed conversion; SEM: square error of means. * EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

The feed conversion also showed system × season interactions (Table 5; Figure A7), and in the spring, the systems did not differ. In the summer, the animals in the iCLF system consumed more than twice than those did in the forage one for each kg of LWG in relation to the animals in the iCL system, while the other systems did not differ from each other. In the autumn, the animals grazing the EXT, iCLF and iLF systems consumed an average of 12.5 kg more fodder for each kg of LWG when they were compared to the INT and iCL systems. Finally, in winter, the animals in the iLF system consumed more than twice the forage that was consumed for each kg of LWG compared to the animals in the iCL system. The iCLF and EXT systems were similar to the iLF one as well as the iCL one, while the animals of the latter also had an FC that was equivalent to those of the INT system.

A system × season interaction was also observed for ADG (Table 5; Figure A8). In the spring and autumn seasons, the ADG was similar for all of the systems. In the summer, the animals that grazed in the iCLF system showed lower ADG values when they were compared to the iCL, INT and iLF systems, and there was no difference for the EXT one. The animals from the iCLF and iLF systems during winter expressed lower ADGs than the other systems did, which did not differentiate amongst themselves.

The methane emission which was estimated in relation to the ADG (Table 5; Figure A9) presented a system × season interaction effect. In the summer, the animals of the EXT and INT systems emitted approximately 0.2 kg more enteric CH_4 per kg than they did ADG when they were compared to the animals of the iCL system, whereas the systems with trees were similar to both of the previous ones. In the autumn, the animals in the EXT system emitted higher amounts of CH_4 per kg of ADG when they were compared to the iCL and INT steers, and again, the iCLF and iLF systems were equivalent to the rest of them in this same season (Figure A9).

4. Discussion

4.1. Forage Mass, In Vitro Digestibility and Nutrient Availability

The soil, pasture, and grazing management, as well as the presence of trees, influenced the availability of digestible forage mass and the production of nutrients per treatment. Pastures are complex environments, and a combination of factors impacts on the growth and quality of the grasses in them. In the same area, there is variability in the available material and in the nutrients that are present in each portion that are grazed upon by the animals, so a better way to analyse the components is to relate the chemical characteristics of the pasture to the mass production, thus, obtaining a more global view of the area that is being analysed.

The intermediate production of the forage mass in the systems with trees (iCLF and iLF) in comparison with the intensive systems (without trees) can be explained by the availability of factors such as light energy and/or water. Integrated systems are more complex than those where agriculture, livestock and timber are produced separately [17]. The tree component changes the microclimate of the pasture production system [11], influencing the growth of the forage plants [17] due to the competition for light energy, and available nutrients and water in the soil [29]. Oliveira et al. [30] also observed greater forage availability for Piatã grass when they were grown without trees compared to an integrated livestock–forest system, and Santos et al. [31] concluded that the dry matter availability and the chemical and structural characteristics of Piatã grass are affected under iLF systems.

The intensive and/or integrated systems showed advantages regarding the production of the digestible forage mass and the nutrients per hectare throughout the year in the case of the INT and iCL systems, or for most of the year in the systems including trees. Even for the low input system (EXT), it was demonstrated that the simple careful control of the animal stocking rate can result in higher production rates since the average animal stocking rate was above the national average (1.3 vs. 1.06 AU ha^{-1}, respectively [32]). The production of nutrients and the availability of digestible forage mass in the different treatments followed the same trend as the production of the forage mass, except for NDF, which was lower in the iCL system despite there being higher values for the forage mass. However, this is advantageous and indicative of a better quality of forage.

Sousa et al. [33] found no treatment effects on the availability of digestible forage mass of the shadow effect in the wooded systems, unlike in the present experiment, where the wooded systems had an intermediate digestible forage mass in comparison to intensive and extensive systems. According to Coêlho et al. [34], digestibility may vary according to the species of grass, the degree of shading and the season. These factors, in addition to the production of forage mass, may explain the differences observed in the values of digestible forage mass that are available per evaluated treatment and throughout the seasons in the present study. As in the present study, Santos et al. [31] found that there was a greater availability of digestible forage mass and CP in the treatment of Piatã grass pasture without trees when they were compared to the integrated livestock–forest system.

In southeastern Brazil, the climate is well defined, with the spring and summer usually being hot and humid, while autumn and winter are dry and cold. For this reason, in the dry seasons, there is greater competition for water. This leads to a general drop in the production of forage mass and the nutrients in the dry seasons, and the opposite occurs in the humid seasons, as observed in the present study. According to Paciullo et al. [35], in shaded conditions, the nutritional value of the forage is improved by an increase in protein content and IVDDM, and a reduction in the NDF. This is because the plants under shade have a slower growth rate when they were compared to the plants under the full sun [36], and the maintenance of the vegetative stage for a longer time leads to higher BP values, as observed in the present experiment for the wooded systems. Pandey et al. [37] also report that shading can be favourable to increase the quality of forage, as shade improves leaf elongation, leaf appearance rates and leaf blade length, but it reduces the tillering rate [38].

4.2. Animal Performance

The greater accumulation of digestible forage mass and nutrients, in addition to the better feed conversion that was observed in INT and iCL systems during most of the year was reflected in an FLW that was 14 and 11% higher, respectively, when it was compared to systems with trees, and an FLW that was 11 and 8% higher, respectively, than the EXT system against the earnings per animal and area. Although the DMI was higher in the iCLF system than it was in the EXT one, this factor did not result in a greater LWG by the animals. This was best the INT and iCL systems, even with DMI being equal to those of both the iCLF and EXT systems.

In general, the observed DMI was close to the values that were predicted by Azevêdo et al. [39] and the NRC [40], with them being at 7.8 and 8.7 kg MS day^{-1}, respectively. These data agree with results from Pontes et al. [13] who also observed a higher ADG and stocking rate in the integrated system without trees and with Oliveira et al. [41], who reported that the LW, ADG and stocking rate increased with the intensification of the grazing systems, in the same way as that which was observed in the INT and iCL systems in the present study. Sakamoto [42], as in the present experiment, observed significant effects of intensification on DMI, ADG and FC when they were comparing the pasture systems with different levels of intensification to an extensive low stocking system.

The iCLF and iLF systems were associated with a lower forage mass production in winter and the lowest ADG and LWG ha^{-1}. This is in contrast to observations by Oliveira et al. [30] and Santos et al. [31], who found no differences in the ADG of the animals that were raised in an integrated livestock–forest system compared to those grazing areas without trees. With the increase in the proportion of trees in the system, and consequently, the shading effect, there was a reduction in the forage density [31], as observed in the present trial. The tree component in the cattle grazing systems protects the animals from the wind and extreme temperatures [43]. The inclusion of trees in the pasture systems in the present study resulted in a positive effect on environmental conditions and on forage growth, but it resulted in lower animal performance when it was compared to the systems without trees, as also reported by Pontes et al. [13].

The iCL and iCLF systems had the lowest stocking rates (AU ha^{-1}) during the summer when they were compared to the INT and iLF ones due to the reduction in available land area (by 1/3) for the animals for the growth of maize and the subsequent renewal of the pasture. This is an inherent component of the integrated production systems, and it is interesting to highlight that 34.7 and 26.6 t ha^{-1} of maize [17] was produced in the iCL and iCLF systems, respectively, and 35.7 m^3 ha^{-1} of wood was produced (unpublished data) in the iCLF system. Although they are associated with the lowest stocking rates in summer, the integrated agricultural systems can generate additional income for farmers, such as maize and wood for the iCLF system. This argument of the trade-off benefits is supported by Salton et al. [44] who conclude that the systems integrated with maize also perform better in terms of the beef production due to the positive effects of rotation between cropping and livestock production.

In the integrated maize cropping systems in the present study, the maize was harvested for silage. However, the silage that was produced was not offered to the animals of those same systems, therefore, the overall availability of forage, and consequently, the stocking rate, were reduced. Hypothetically, it would be expected that if the animals were supplemented with the maize silage at that time, the stocking rate and the animal performance would be higher than those that were observed, and this is an important consideration when one is comparing the systems.

In terms of the animal performance, the integrated systems with trees (iCLF) had 10.7% lower final LWs when were compared them to the integrated systems without trees (iCL). As previously described, the probable shading effect of the trees, which reduced growth, and consequently, the accumulation of forage, was directly reflected in the LWG response. Pontes et al. [13] evaluated the performance of heifers in integrated agricultural systems (with and without trees) with two levels of supplementation during the winter, and they reported a reduced ADG and stocking rate for the production systems that were integrated with trees in comparison to those that were in full sun, as observed in the present study. The iCLF and iCL systems had an annual support capacity of 70 and 98%, respectively, which are above the average for Brazil in 2019 [32]. This demonstrates the productive potential of these systems since in addition to livestock, there is the production of crops and wood as previously mentioned.

4.3. Emission of Enteric Methane

The average CH_4 emission per animal in the present experiment was 202.6 g day^{-1}, a value that is numerically above that which was predicted by the Tier 1 methodology that was proposed by the IPCC [45], of 156.0 g day^{-1}. The differences observed in relation to the CH_4 emission per kg of DM intake are directly linked to the differences that were observed in the DMI since there were no significant differences between the emissions per animal per day.

According to Buddle et al. [46], the consumption of dry matter is the most important modifier of methane emissions in ruminants, thus, the methane emission per unit of DM intake can increase or decrease according to the level of intake, which can be explained by a greater ruminal turnover, leading to less digestibility of the ingested mass. Sakamoto [42] compared the intensification levels and the emission intensities and found no significant difference in the annual averages between the treatments, as observed in the present experiment, but they did find seasonal differences between the treatments.

Several studies have demonstrated the influence of pasture quality on CH_4 emission, with increasing forage digestibility being identified as a potential mitigation option [47]. According to Pinares-Patiño et al. [48], CH_4 emission and LW are positively correlated, which can be seen in the higher final weight and higher emission of animals in the INT system. However, according to Mercadante et al. [49], there is no evidence that the animals with a higher efficiency produced less enteric methane, even if they have a lower DMI and performance which was equivalent to that of the animals that were considered to be inefficient.

In relation to the differences that were observed in methane emissions per kg of ADG and due to the system × season interaction, in summer, the EXT and INT systems due to the low ADG presented in both in this same season, the emission was about 0.2 kg greater in comparison to the iCL system, with the systems with a tree component being intermediate to the others in this season. In the autumn, the EXT system had the highest value, resulting from a low ADG (but this was not different from the other systems except iCL) and an average emission (similar to iCL and iLF). The animals in this system emitted about 230 g of methane more than they did in the INT and iCL systems to gain the same weight. The tree systems, again, did not differ from the other two intensified systems or from the EXT one along this variable.

The studies by Pontes et al. [13] and Sakamoto [42] that analysed the levels of the intensification of pastures and integrated systems with and without trees found no differences between the treatments in the CH_4 emissions per animal or per kg LW, but they did report significant differences between the seasons or periods in the emissions in relation to ADG caused by the indirect effects of performance (ADG and stocking rate). As in the present work, these same authors observed a higher emission per kg ADG when the ADG was lower than that of the intensive [42] and/or integrated systems [12].

The CH_4 emission intensity indicator, (gCH_4 $kgADG^{-1}$) (LWG ha^{-1})$^{-1}$ $year^{-1}$, has been demonstrated as a useful metric as it can show the CH_4 emission intensity in relation to the efficiency of the gain per area of each system. The evaluation of the CH_4 emissions in the animals that were raised on a pasture is always a challenging process in terms of the available techniques and the analysis and interpretation of the results. Often, the experimental units that are available for comparison limit the ability to discern significant treatments effects. For this reason, it is important to interpret the available variables and compare them with those that are relevant, and they can show the productive characteristics in relation to the emissions that are being assessed. It was evident that the intensification and use of sustainable technologies in the pasture systems reduce the intensity of the CH_4 emissions per kg of the product that is produced.

The higher emission intensity in kg of CH_4 which was emitted in comparison to the amount of carcasses that were produced (kg of carcass eq. ha^{-1}) in the EXT system was probably the due to the lower availability of forage mass, nutrients and the lower DMI, which led to a lower ADG and final weight, even though there was no difference in the emission of CH_4 per animal.

The intensification of the pasture management in order to improve the feed efficiency or productivity, using better quality fodder and animals with an improved genetic merit, can reduce the methane emissions per product unit [50], with a smaller production area and a shorter time until slaughter, which can avoid the opening of new forest areas for pasture [51]. For the sustainable intensification of pastures, it is important to also consider the benefit of animals on the plants and the soil since grazing cattle are the catalysts, recycling plant material and modifying nutrient dynamics [11].

According to Oliveira Silva et al. [52], the recovery of degraded pasture areas has the greatest potential for GHG mitigation due to carbon sequestration. Pontes et al. [13] estimated that the potential for sequestering C from woody biomass at a density above 159 trees per ha more than compensates for the increase in the emissions per unit area, which makes the integrated systems of animal production with trees a promising CO_2 mitigation strategy in subtropical regions. In the present experiment, the animals from the intensified and integrated systems produced more kg of carcass per unit area, and consequently, less CH_4 per kg of carcass was produced in the pastures with soils with a greater potential for C sequestration, as indicated by Oliveira Silva et al. [50].

5. Conclusions

Beef cattle that were raised in an intensive and/or integrated pasture production systems have access to a greater forage mass and nutrients than those that are raised extensively. Pasture systems that receive management such as soil pH correction and fertilization, rotational grazing or are integrated with maize cropping can produce animals with higher final weights and average daily gains, which dilutes the enteric CH_4 emissions per kg weight gain. In these same systems and even in those that are integrated with the forest component, a greater efficiency in the gain per area is obtained, however, as the complexity of the components within the same area increases, as in the integration of livestock farming and forestry, the gain per unit area is comparable to the systems with the extensive management and control of the stocking. This same pattern is observed in the intensity of methane emission in relation to the efficiency of the animal gain by area. Higher yields and carcass qualities are obtained from animals in the pasture production systems that have been intensified and/or integrated with maize cropping. Animals produced in the intensive pasture and integrated systems emit less methane per kg of carcass produced than those that are raised in extensively managed systems.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

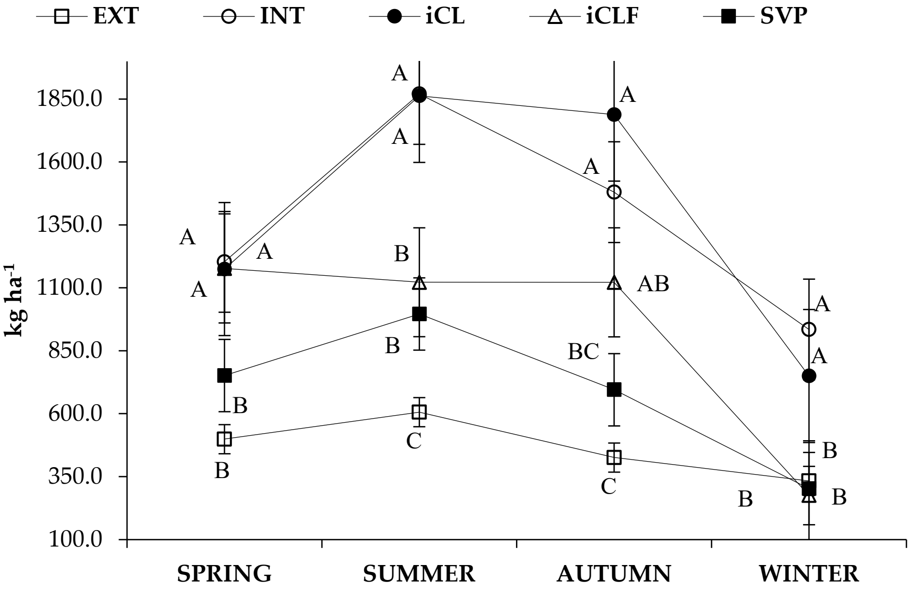

Figure A1. Dry mass availability during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

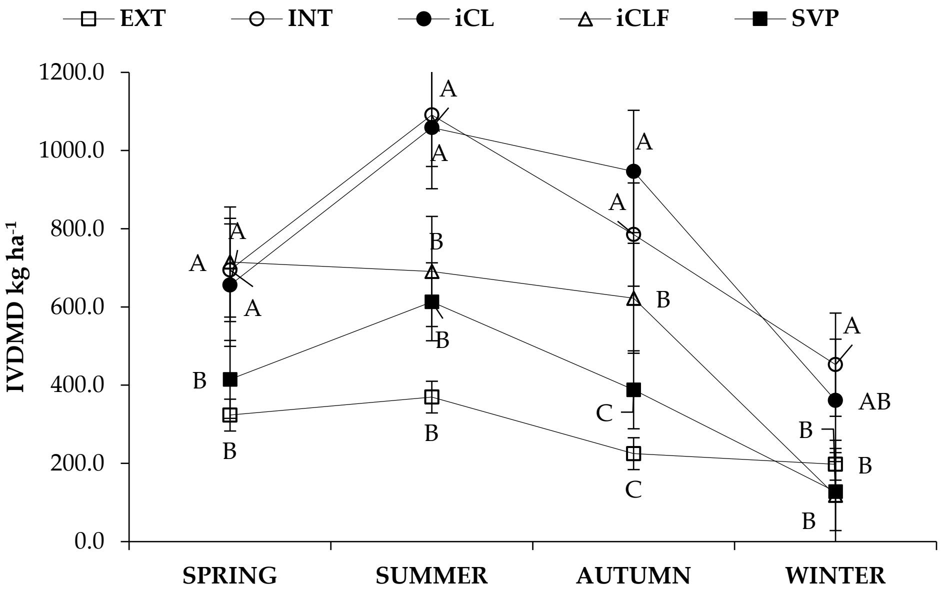

Figure A2. In vitro digestibility (kg ha^{-1}) during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

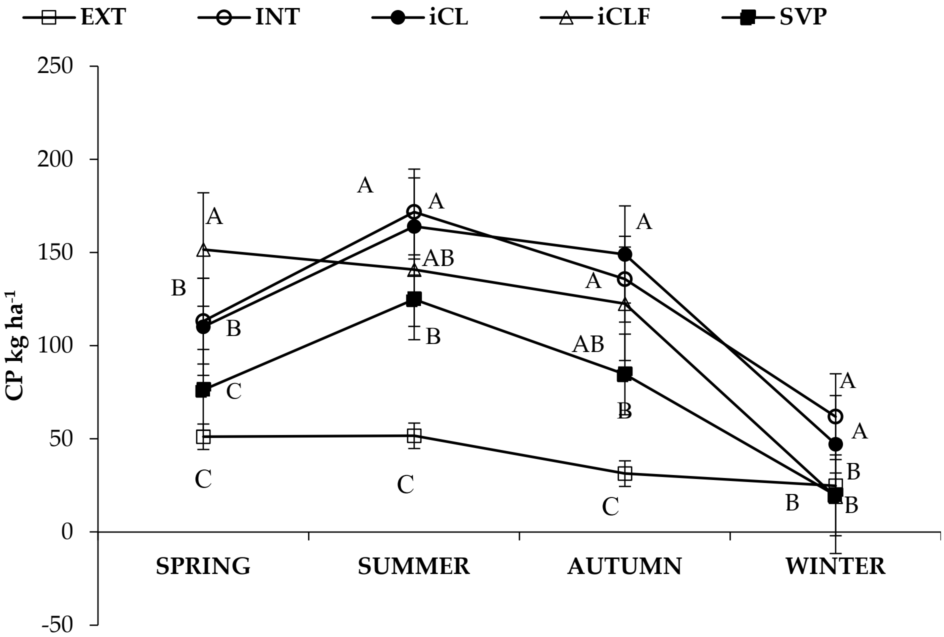

Figure A3. Crude protein (kg ha^{-1}) during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

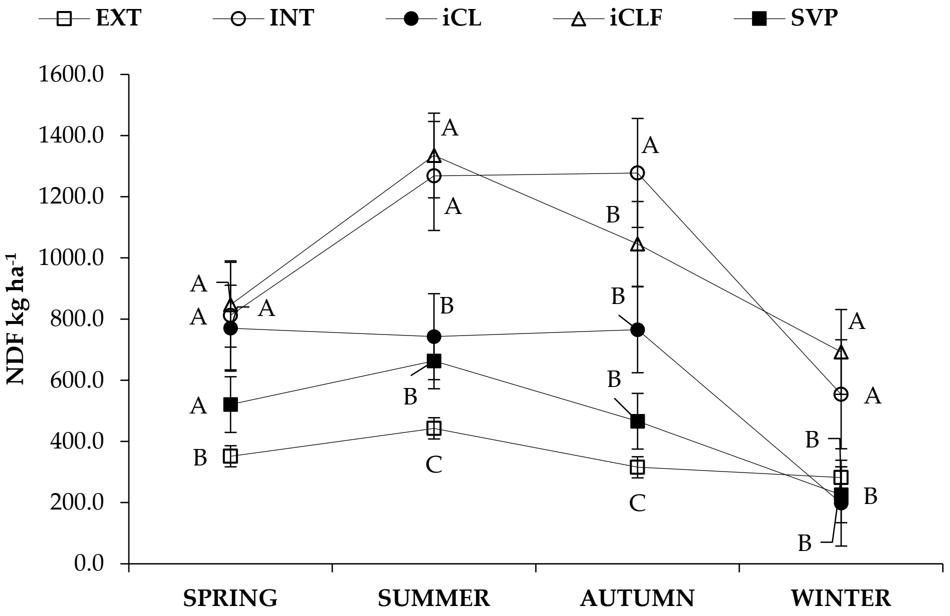

Figure A4. Neutral detergent fibre (NDF) (kg ha^{-1}) during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

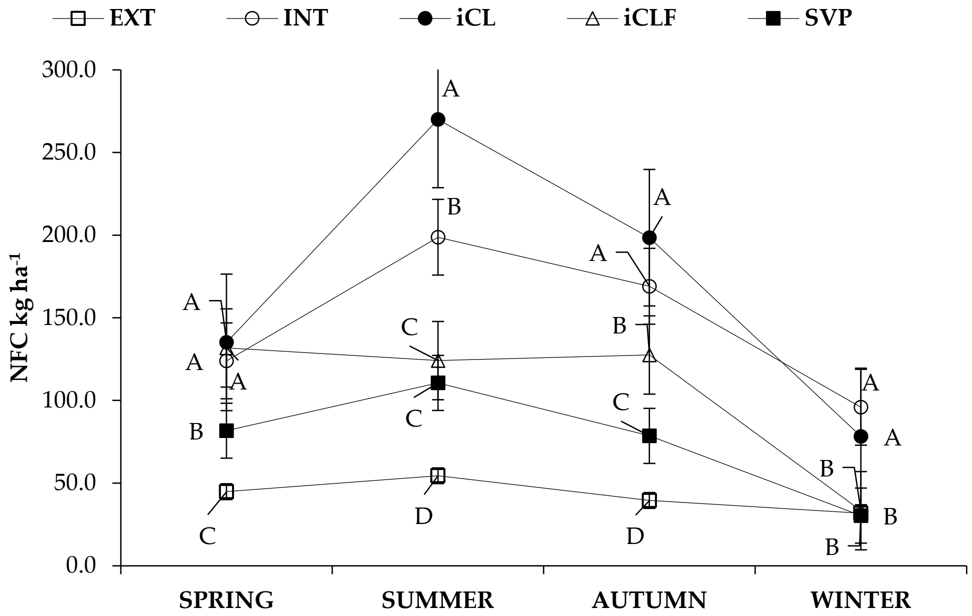

Figure A5. Non-fibrous carbohydrates (kg ha^{-1}) during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

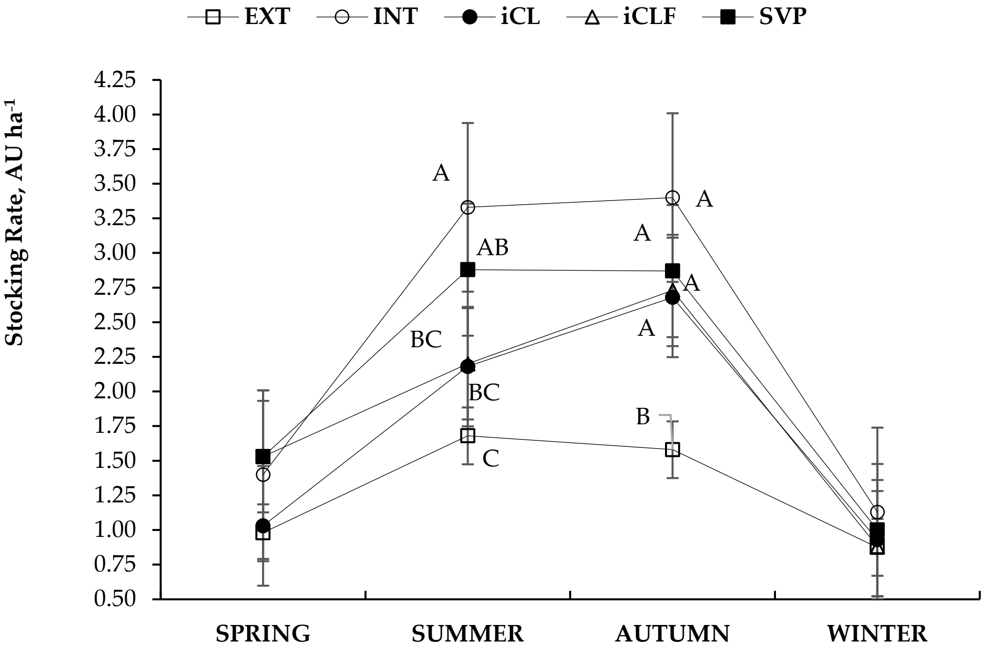

Figure A6. Stocking rate (AU ha^{-1}) during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

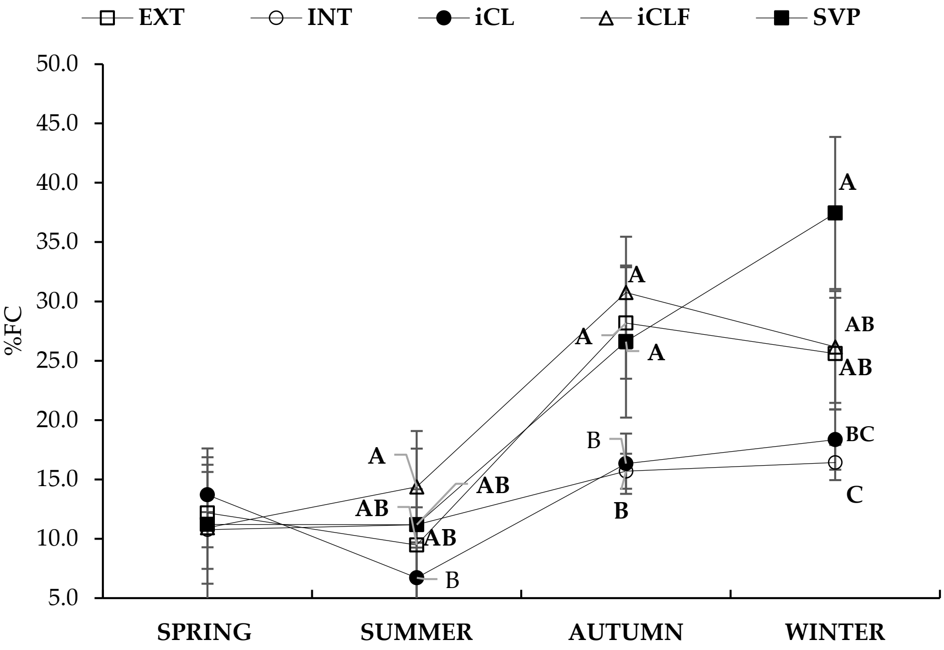

Figure A7. Feed conversion (in %) during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

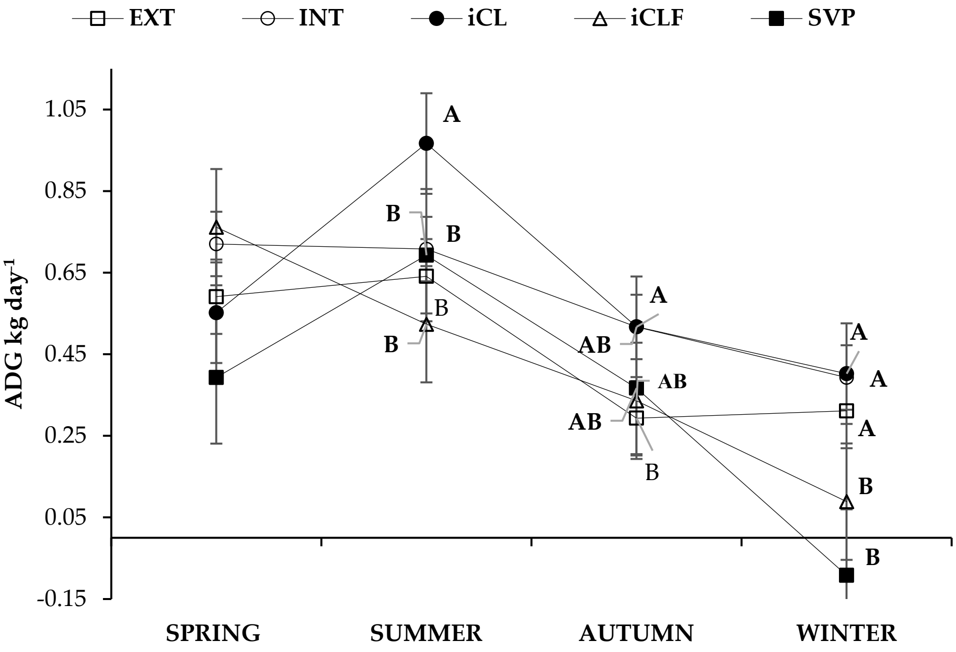

Figure A8. Average daily gain (kg) during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

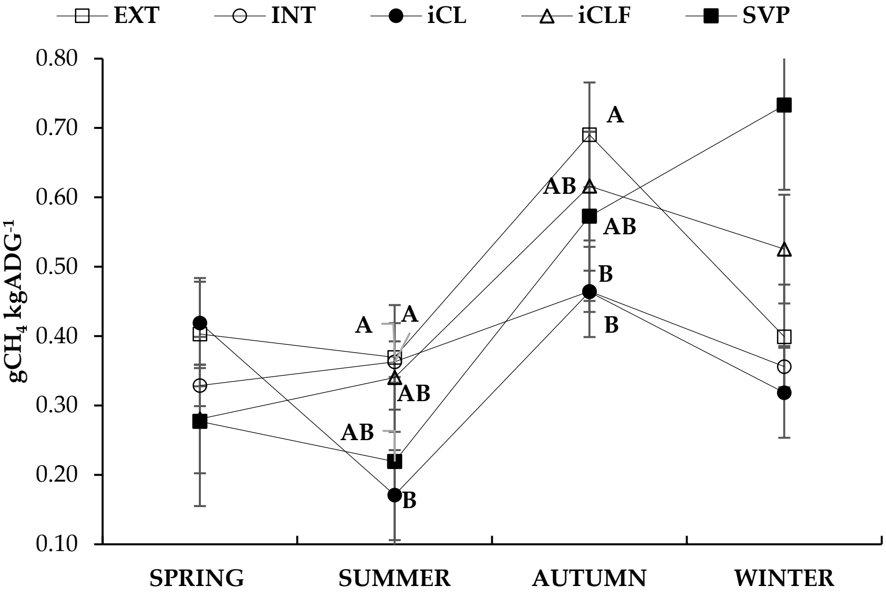

Figure A9. Enteric methane emission (kg) per kilogram of ADG during seasons from different livestock production systems (means of two years). Different letters in the same season differ from each other ($p < 0.05$) according to the Tukey test. EXT: extensive; INT: intensive; iCL: integrated crop–livestock; iCLF: integrated crop–livestock–forest; iLF: integrated livestock–forest.

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