



## Confinement and semi confinement of lambs in an integrated production system: impacts on production performance, infection by gastrointestinal nematodes, and selected blood analytes

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**ABSTRACT:** This trial evaluated the production performance, gastrointestinal nematode infection (GIN), and selected blood analytes, in Corriedale lambs during the finishing phase in an integrated crop-livestock system (ICLS) with 2 stocking rates. Fifty-two Corriedale lambs were divided into 4 groups: a semi confinement system with a low stocking rate (T1) for 70 days; a semi confinement system with a high stocking rate (T2) for 70 days; confinement for 28 days, followed by semi confinement with a low stocking rate (T3) for 42 days; and confinement for 28 days, followed by semi confinement with a high stocking rate (T4) for 42 days. Body weight, dry matter intake, blood collection, and faecal analysis of nematode eggs were performed for each animal. The stocking rates did not influence body weight gain for the semiconfined lambs (T1 and T2) or for the confined/semiconfined lambs (T3 and T4). All treatments had lower EPG counts at the end of the experimental period. The nematode genera identified during the experiment trial were *Haemonchus* and *Trichostrongylus*. There were no significant differences ( $P > 0.05$ ) for packed cell volume, total plasma protein, and eosinophil means between treatments. These results suggested that the confinement followed by semi confinement in an ICLS, was an option for lamb production during the finishing phase which could be useful in reducing GIN without affecting the production performance.

**Key words:** integrated crop-livestock system, *Haemonchus*, *Trichostrongylus*, sheep.

## Confinamento e semiconfinamento de cordeiras em um sistema integrado de produção: impactos na produção, infecção por nematódeos gastrointestinais e em variáveis sanguíneas

**RESUMO:** O objetivo deste experimento foi avaliar o desempenho produtivo, infecção por nematódeos gastrointestinais (GIN) e variáveis sanguíneas selecionadas, em cordeiras Corriedale durante a fase de terminação em sistema integrado lavoura-pecuária (ICLS) com duas taxas de lotação. O experimento utilizou 52 cordeiras Corriedale divididas em quatro grupos: sistema de semiconfinamento com baixa taxa de lotação por área (T1) por 70 dias; sistema de semiconfinamento com alta lotação por área (T2) por 70 dias; confinamento por 28 dias, seguido de semiconfinamento com baixa lotação por área (T3) por 42 dias; e confinamento por 28 dias, seguido de semiconfinamento com alta lotação por área (T4) por 42 dias. Peso corporal, ingestão de matéria seca, coleta de sangue e análise fecal para ovos de nematódeos foram avaliados para cada animal. As taxas de lotação não influenciaram no ganho de peso dos cordeiros semiconfinados (T1 e T2) ou nos confinados/semiconfinados (T3 e T4). Todos os tratamentos apresentaram contagens menores de EPG no final do período experimental. Os gêneros de nematódeos identificados durante o experimento foram *Haemonchus* e *Trichostrongylus*. Não houve diferença significativa ( $P > 0.05$ ) para hematócrito, proteína plasmática total e média de eosinófilos entre os tratamentos. Esses resultados sugerem que o confinamento seguido de semiconfinamento em ICLS pode ser uma opção para a produção de cordeiras na fase de terminação e pode ser útil para a redução da GIN sem afetar o desempenho da produção.

**Palavras-chave:** sistema integrado lavoura-pecuária, *Haemonchus*, *Trichostrongylus*, hematócrito, ovinos.

## INTRODUCTION

In sheep farming, pasture management could be an alternative to helminth control by reducing the ingestion of infective larvae by the host (ROCHA et al., 2008), as the type of animal raising system influences parasite burden and impacts animal production (VIEIRA et al., 2018; PARIZ, 2017). In Brazil, *Haemonchus contortus*

and *Trichostrongylus colubriformis* are the main gastrointestinal nematodes (GIN) infecting sheep, especially in an extensive system (CARVALHO et al., 2021; WILMSEN et al., 2014).

A fundamental requirement for agricultural sustainability focusing on rural producers is the reduction of production costs, which can be achieved through the annual use of agricultural land, including crop cultivation and livestock production (PARIZ et al.,

2009). The integrated crop-livestock systems (ICLS) seeks synergism between productions of annual crops and livestock forage, promoting soil-plant-animal interactions diminishing the contamination of pasture by infective larvae of gastrointestinal nematodes, and as an alternative for controlling worms in sheep (MORAES et al., 2014; PARIZ et al., 2017).

ALMEIDA et al. (2018) reported that a way to eliminate the nematode parasite-free living stages would be to implement an ICLS, known as integrated agricultural and livestock production systems, in which ecological interactions among the different land-use promotes areas with different soil covers, generating different microclimates, thus influencing, and breaking the free-living stages of GI parasite's cycles. Thus, enhancing animal performance since a low parasite burden is related to better weight gain (CARVALHO et al., 2021).

Confinement as an intensification of the sheep production system reduces the helminth burden providing an increase in food consumption expressed in dry matter (CARVALHO et al., 2007), improving animal performance (ALMEIDA et al., 2018; VIEIRA et al., 2018; AMARANTE et al., 2004) and reducing the production cycle or breeding (MAESTÁ et al., 2010). Therefore, the strategy of confining the animals before an ICLS semi confinement could minimize the effects of parasitism and improve animal performance. Moreover, it allows the farmer to determine the appropriate time point for the entry of animals into the system, which is determined by the optimal time for forage management. Thus, the purpose of this trial was to evaluate changes in GI parasite burden, body weight, weight gain, dry matter intake, and selected blood analytes (packed cell volume, total plasma protein, and the percentage of eosinophils) during the finishing phase in Corriedale lambs naturally infected by GIN in an ICLS with two stocking rates in confinement and semi confinement.

## MATERIALS AND METHODS

The experiment was conducted at the Lageado Experimental Farm, located in Botucatu, São Paulo, Brazil (48°25'28" W, 22°51'01" S, and 777 meters above sea level).

The preparation of the experimental area began in November 2016, with previous desiccation of the plants present in an integrated agricultural production system area, which had been conducted for five years. In December 2016, corn (hybrid 2B587 Power precocious) was sown intercropped with marandu palisade grass. In April 2017, corn

was harvested for silage production, followed by the overseeding of black oat (*Avena strigosa* Schreb cv. Garoa). In June, the experimental period began with the introduction of lambs in the area and ended in September 2017, totalling 70 days. During the experimental period, climate data were measured daily, calculating the monthly means of each attribute.

The study population comprised 52 lambs of the Corriedale breed, originating from a commercial farm and naturally infected with gastrointestinal nematodes. The animals were separated into 4 groups of 13 animals each with a mean initial body weight of 23.8 kg  $\pm$  2.61. The experiment was a randomized complete block design with repeated measures over time. Blocking was organized regarding body weight, allocating the animals by casting lots in the treatments, with the animal being considered as an experimental unit in the following treatments: T1) Semi confinement with a low stocking rate per area (686.4 kg of body weight/hectare) for 70 days; T2) semi confinement with a high stocking rate per area (1113.6 kg of body weight/hectare) for 70 days; T3) confinement for 28 days, followed by semi confinement with a low stocking rate per area (686.4 kg of body weight/hectare) for 42 days; and T4) confinement for 28 days, followed by semi confinement with a high stocking rate per area (1113.6 kg of body weight/hectare) for 42 days.

The stocking rates ranged according to the size of the paddocks. Thus, for the lower stocking rate, 13 lambs (312 kg) were allocated in a 4,544 m<sup>2</sup> paddock (T1 and T3), and for the highest stocking rate, 13 lambs (312 kg) were allocated in a 2,800 m<sup>2</sup> paddock (T2 and T4), totalling 686.4 kg. hectare<sup>-1</sup> and 1,113.6 kg hectare<sup>-1</sup>, respectively. The paddocks were delimited with an electrified four-wire fence, and water and shades were also available for thermal comfort. The animals were kept on pasture during the day, and in the late afternoon, they were returned to a covered shed with thermoacoustic tiled roof, side curtains and a clay floor lined with rice husk, where they were housed and received the supplement in a trough in collective stalls in the respective treatments. With the decrease in pasture availability, in the final third of the experimental trial, the animals received one-third of the supplement in the pasture and two-thirds in the shed.

The roughage: concentrate ratio was calculated based on pasture forage availability and on the amount of silage produced in the area. Diet was estimated for an average daily weight gain of 200 g in semi confinement and 250 g in confinement (NRC, 2007) to meet the demands of growing lambs. The

diet was formulated via the computer software Small Ruminant Nutrition System (SRNS) based on the Cornell Net Carbohydrate and Protein System for sheep (FOX et al., 2004). The supplement was provided in troughs, and the animals always had free access to water.

The period of adaptation of the lambs to the diet, both in the pasture and in the confinement, was four days (23 to 27 June), and later, on 28 June, the animals were individually weighed using a mobile digital scale with a rectangular cage 1.30x0.60 m (COIMMA® – Model ICS-300) to start the trial.

Six days before starting the experiment, faecal and blood samples were taken from all animals to determine eggs per gram of faeces (EPG) and packed cell volume (PCV). Subsequently, animals with an EPG of higher than 4,000 and a PCV of lower than 21% were identified, following recommendations by AMARANTE et al. (2004) and were treated with closantel (10 mg/kg BW, Diantel®, Brazil). Nine days later, the efficacy of closantel treatment was calculated by EPG reduction (R-EPH): R-EPG (%) = 100 (1 – EPG means after treatment/EPG means before treatment) for those lambs.

Faecal and blood samples were taken from all lambs at every 2 weeks (6 different time points) and body weight was recorded on the same occasion. The dry matter intake was estimated based on the amount of supplement provided and the surplus for each treatment, stipulating the individual intake by the percentage of body weight of each animal.

Faecal egg counts were performed using the modified McMaster technique (GORDON & WHITLOCK, 1939), following the specifications for small ruminants. The coprocultures (ROBERTS & O'SULLIVAN, 1950) were performed in a pool of samples from all the animals to identify genera of third-stage infective larvae (L3) (UENO & GONÇALVES, 1998).

Blood samples were obtained by jugular vein puncture in tubes with EDTA (BD Vacutainer® K2 EDTA Blood Collection Tube; Becton, Dickinson and Company, USA), to perform the PCV, total plasma protein (TPP), and the percentage of eosinophils for every animal. Packed cell volume was determined by the microhematocrit method, and (TPP) concentrations were determined with a hand-held refractometer. The refractometry measurements were obtained with an Atago-type non-temperature-compensated refractometer, all readings were made at room temperature (approximately 25°C), and the percentage of eosinophils was determined by differential leukocyte counting in Wright-stained blood smears (JAIN, 1986).

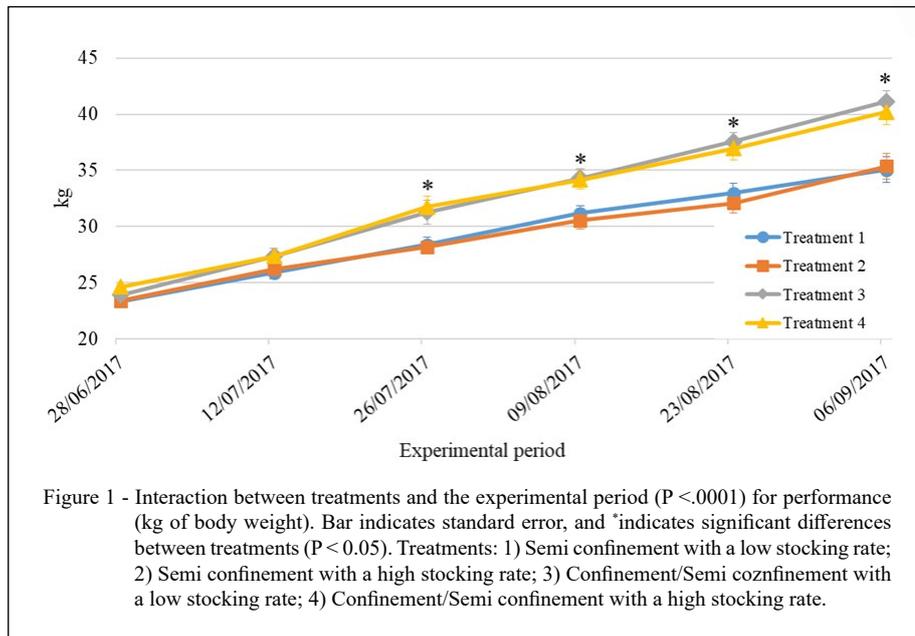
All data were initially evaluated for normality using the Shapiro–Wilk test from the PROC UNIVARIATE procedure in SAS (SAS Institute version 9.4, USA). All data were considered normally distributed when  $W \geq 0,90$ . The data that did not meet the normal distribution criteria were transformed using  $\log(x+1)$  prior to the analyses. 'Animal' was considered as the experimental unit for all variables, except for dry matter intake, in which each pen was considered as the experimental unit. Performance and daily ratio intake data were analysed using the SAS PROC MIXED and EPG, PCV, total plasma protein, eosinophils, and the interactions between treatments and time points were analysed using the PROC GLIMMIX procedures. The covariate used in the model was "un." Error bars are presented as the mean standard error (MSE) and were analysed using the SAS PROC MEAN procedure. The means were adjusted by the least square means for multiple comparison analyses ( $P \leq 0.05$ ).

## RESULTS

There were significant differences for final body weight ( $P = 0.0001$ ), mean weight gain ( $P < .0001$ ), and daily mean weight gain ( $P < .0001$ ) between T1 and T2 treatments when compared with T3 and T4 (Figure 1). There were no significant differences in initial body weight between treatments ( $P = 0.1929$ ), demonstrating homogeneity (Table 1). The animals kept in confinement for 28 days, followed by semi confinement, had higher weight gain and were consequently heavier by the end of the trial.

The performance of the confined lambs (Table 1) that were evaluated at every 2 weeks and later went to semi confinement (T3 and T4) suggested that they continued to gain weight when compared to the animals that remained in a semi confinement system throughout the trial (T1 and T2). There were no significant differences for the studied stocking rate systems.

For dry matter intake (Table 1), there were significant differences between the same treatments ( $P < .0001$ ), in which the confined animals ingested a higher amount of supplement per day (T3 and T4) than the semiconfined animals (T1 and T2). There was no influence of semi confinement stocking rates in dry matter intake. The consumption of supplements by the animals in T3 and T4 during the 28-day period that they were confined and, subsequently, the 42-day period that they were semiconfined, was on average 0.975 kg and 1.091 kg ( $P = 0.0004$ ) in the confinement period and 1.059 kg and 1.104 kg ( $P = 0.3411$ ) in the



semi confinement period, respectively. There were no significant differences between the time points for T3 ( $P = 0.0994$ ) and T4 ( $P = 0.7215$ ).

Anthelmintic treatment was chosen for animals with EPG higher than 4,000 and PCV lower than 21% in five lambs of T1, four lambs of T2, four lambs of T3, and three lambs of T4, totalling 16 lambs. After nine days the R-EPG was 89.3% for closantel efficacy for these lambs (data not shown). The lambs from T1 and T2 had significantly ( $P = 0.0008$ ) higher EPG counts when compared with T3 and T4 lambs (Table 1). Although, there were no significant differences for EPG counts at the beginning ( $P = 0.9736$ ) or at the end ( $P = 0.2131$ ) of the experimental period between treatments, the confined animals had lower EPG than the semiconfined animals, without significant differences between stocking rates. Confined lambs of T3 and T4 after 14 days showed a significant reduction in EPG counts compared to EPG counts at the beginning of the trial while T1 and T2 showed a significant EPG reduction only after 42 days.

The third-stage larvae (L3) identified in coprocultures at the beginning of the experiment in order of prevalence were *Haemonchus* (94.5%) and *Trichostrongylus* (5.5%). At the end of the trial, the identified L3 were *Haemonchus* (73.1%), *Trichostrongylus* (23.3%), and *Cooperia* (3.9%). The means of the nematode genera for the treatments were: T1: 86.1%, 11.4%, and 2.5%; T2: 90.3%, 6.3%, and 3.3%; T3: 79.6%, 17.7%, and 2.8%, and T4: 78.7%, 18.9%, and 2.4%, respectively, for *Haemonchus*,

*Trichostrongylus* and *Cooperia*.

The initial and final means for PCV, total plasma protein, and eosinophils are shown on table 2. There were no significant differences ( $P > 0.05$ ) for PCV, TPP, and eosinophil means between treatments along the experimental trial.

## DISCUSSION

In this study, the final body weight of confined and later semiconfined animals was higher than that of semiconfined animals. The onset of puberty varies according to body weight, which is between 33 kg and 42 kg, in agreement with other reproductive parameters, such as age, body condition score, the presence of oestrus, or even the quantification of progesterone (BOULANOUAR et al., 1995; MORI et al., 2006; FERRA et al., 2010; DANTAS et al., 2016). Therefore, the lambs of all treatments in the present study could be destined for reproduction, as they had adequate weight and age. Under the experimental conditions of the study reported by GARCIA et al. (2010), crossed breed animals in an intensive system gained an average of 0.202 kg per day, while animals in a semi-intensive system gained an average of 0.109 kg per day, which was below the gain observed in the present study in both systems. CAVINI et al. (2015) reported a daily average weight gain of 0.225 kg in lambs until fattening in pens, which was similar to the performance of confined animals.

Table 1 - Performance, dry matter intake, and eggs per gram of faeces of lambs kept under confinement and semi confinement and two stocking rates in an integrated crop-livestock system.

| Variables                         | Treatments <sup>6</sup> |                  |                  |                  | MSE <sup>5</sup> | P      |
|-----------------------------------|-------------------------|------------------|------------------|------------------|------------------|--------|
|                                   | 1                       | 2                | 3                | 4                |                  |        |
| Initial body weight (kg)          | 23.4                    | 23.4             | 23.9             | 24.6             | 0.6              | 0.1929 |
| Final body weight (kg)            | 35.1a                   | 35.4a            | 41.1b            | 40.2b            | 1.5              | 0.0001 |
| WG <sup>1</sup> average (kg)      | 11.7a                   | 11.9a            | 17.2b            | 15.6b            | 1.2              | <.0001 |
| DWG <sup>2</sup> average (kg/day) | 0.16a                   | 0.17a            | 0.24b            | 0.22b            | 0.02             | <.0001 |
| SCD <sup>3</sup> (kg/day)         | 0.7a                    | 0.8a             | 1.1b             | 1.1b             | 0.04             | <.0001 |
| EPG <sup>4</sup> average          | 4,006a (Log 3.2)        | 4,256a (Log 3.3) | 2,368b (Log 2.7) | 2,849b (Log 2.6) | 817.1            | 0.0008 |
| EPG <sup>4</sup> initial          | 8,931 (Log 3.7)         | 8,762 (Log 3.8)  | 9,016 (Log 3.7)  | 11,191 (Log 3,7) | 3,067.3          | 0.9736 |
| EPG <sup>4</sup> final            | 700 (Log 2.4)           | 900 (Log 2.6)    | 354 (Log 2.0)    | 391 (Log 1.8)    | 255.8            | 0.2131 |

Means followed by the same lowercase letter within a line did not differ significantly ( $P > 0.05$ ); <sup>1</sup>WG: Weight Gain; <sup>2</sup>DWG: Daily Weight Gain; <sup>3</sup>SCD: Supplement consumption expressed in dry matter; <sup>4</sup>EPG: Eggs Per Gram of Faeces; <sup>5</sup>MSE: Mean standard error. <sup>6</sup>Treatments: 1) Semi confinement with a low stocking rate; 2) Semi confinement with a high stocking rate; 3) Confinement/Semi confinement with a low stocking rate; 4) Confinement/Semi confinement with a high stocking rate.

The stocking rates did not influence body weight gain for the semiconfined treatments (T1 and T2) or for the confined/semiconfined lambs (T3 and T4). These results corroborated those reported by ANIMUT et al. (2005), in which the final weight of sheep at different stocking rates did not differ significantly. Lambs confined for the first 28 days gained more weight than semiconfined lambs did, as these animals had a higher supplement intake and a lower energy expenditure. It was also possible to observe that the dry matter intake of the animals

during the confined period and the semiconfined period (T3 and T4) were similar.

The EPG counts were lower in confined lambs than in those of the treatments in semiconfined animals, with no difference between stocking rates. Both systems were beneficial for reducing the parasite burden. Confined lambs had a faster reduction in parasite load as the EPG counts were lower than semiconfined lambs. Nevertheless, all treatments had lower EPG counts at the end of the experimental period, suggesting a decrease of GI parasites

Table 2 - Blood analytes: packed cell volume (PCV), total plasma proteins (TPP), and eosinophil counting (EC) of lambs kept under confinement and semi confinement and two stocking rates in an integrated crop-livestock system.

| Analytes            | Treatments <sup>5</sup> |      |      |      | MSE <sup>4</sup> | P      |
|---------------------|-------------------------|------|------|------|------------------|--------|
|                     | 1                       | 2    | 3    | 4    |                  |        |
| PCV1 average (%)    | 30.8                    | 31.1 | 32.2 | 33.0 | 0.985            | 0.1280 |
| PCV1 initial (%)    | 29.0                    | 28.1 | 28.3 | 29.1 | 2.665            | 0.9751 |
| PCV1 final (%)      | 33.0                    | 33.8 | 34.8 | 35.4 | 1.045            | 0.1370 |
| TPP2 average (g/dL) | 6.0                     | 6.0  | 6.2  | 6.1  | 0.146            | 0.7592 |
| TPP2 initial (g/dL) | 5.6                     | 5.4  | 5.7  | 5.7  | 0.510            | 0.9216 |
| TPP2 final (g/dL)   | 6.2                     | 6.3  | 6.4  | 6.3  | 0.157            | 0.6370 |
| EC3 average (%)     | 1.7                     | 1.7  | 2.2  | 2.9  | 0.493            | 0.0909 |
| EC3 initial (%)     | 1.7                     | 0.8  | 2.8  | 3.2  | 1.000            | 0.1159 |
| EC3 final (%)       | 2.6                     | 2.8  | 2.5  | 2.6  | 1.148            | 0.9229 |

Means followed by the same lowercase letter within a line did not differ significantly ( $P > 0.05$ ). <sup>1</sup>Packed cell volume (PCV), <sup>2</sup>Total plasma proteins (TPP), and <sup>3</sup>Eosinophil counting (EC). <sup>4</sup>MSE: Mean standard error. <sup>5</sup>Treatments: 1) Semi confinement with a low stocking rate; 2) Semi confinement with a high stocking rate; 3) Confinement/Semi confinement with a low stocking rate; 4) Confinement/Semi confinement with a high stocking rate.

infection, regardless of the finishing strategy. According to ALMEIDA et al. (2018) and following UENO & GONÇALVES (1998), the parasite burden was reduced to a degree of infection considered moderate. In addition, confined animals had a lower incidence of gastrointestinal helminths, increasing performance and reducing mortality (ALMEIDA et al., 2018; AMARANTE et al., 2004). The reduced EPG of animals kept in semi confinement could be attributed to the absence of free-living worms in the ICLS (ALMEIDA et al., 2018) and the environmental conditions during the experimental trial. To keep eggs viable in the faeces for later larval development, moist soil, frequent rains, and shade of vegetation are required, as desiccation can be fast when exposed to the sun, causing the death of larvae (AMARANTE, 2014). The low rainfall during the experimental trial may have contributed to the reduction of free-living infective larvae in the pasture, both due to the lower hatching and the low ground cover due to the low regrowth of the oat pasture, leaving the worms more exposed to the sun. In addition, in August, with the rise in environmental temperatures, the faeces were exposed to heat, leading to the desiccation and death of the larvae. Another key factor that reduces the EPG count is the nutritional quality of the diet which improves the body immune response against parasites (AMARANTE, 2014; PARIZ et al., 2017). In this study, the diet was formulated and balanced according to the nutritional and physiological requirements of the lambs.

The nematode genera identified in faecal cultures during the experiment trial were *Haemonchus* and *Trichostrongylus*, the same as those reported by ALMEIDA et al. (2018) in an ICLS, with analogous order of prevalence and according to (WILMSEN et al., 2014), these are most frequent nematode genera identified in the southeast region of Brazil.

The absence of significant differences for PCV, TPP, and the percentage of eosinophils for the two stocking rates (686.4 kg/ha and 1,113.6 kg/ha) is of relevance, suggesting that the ICLS for these lambs allowed the higher stocking rates, decreasing the level of GIN, and did not interfere with the performance of the animals or the dry matter intake.

## CONCLUSION

In our study conditions, the confinement and semi confinement in an ICLS for lambs, regardless of the stocking rate, did not affect the production performance and the dry matter intake. Thus, it could be considered as an efficient option by improving the

reduction of gastrointestinal nematode infection by the frequent parasite genera in the southeast region of the country, attributed to the decrease of free-living worms in the ICLS, without changing the blood analytes. These results showed that there is potential for using the integrated crop-livestock system for the finishing cycle and gaining body weight of lambs.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

## BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The study was approved by the Ethics Committee of the School of Veterinary Medicine and Animal Sciences (CEUA 0001/2017), Universidade Estadual Paulista (FMVZ-UNESP), Brazil and was conducted in accordance with current legislation on animal protection.

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