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Effect of Addition of Turmeric (Curcuma longa) to the Diet on Growth Performance and Serum Lipid Concentration of guinea pigs (Cavia porcellus)

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Abstract

The South American guinea pig rodent has become a livestock animal acceptable for human consumption in different parts of the world. In Ecuador, where originally it has been domesticated by indigenous people, *Cavia porcellus* are widely used as food security due to its meat has similar appearance to rabbit or chicken. Furthermore, Ecuadorian smallholders use local food resources as supplementation as they might provide bioactive compounds with multiples benefits on organic responses. The most used as feed additive for *Cavia porcellus* is Curcuma longa (C. longa) flour, however, several studies have also reported productive, metabolic or immunological responses. Therefore, the aim of the current work has been to evaluate the effect of dietary supplementation of C. longa in Guinea pig. Hereby, 120 animals were randomly distributed into four treatments in a completely randomized design, denominated "Control", "R1", "R2" and "R3" . The first treatment named Control, included a basal diet forage, with a concentrate ratio of 60:40; The second treatment, R1, has been equal to control + 0.60 % of C. longa, while R2, has been like control + 1.30 % of C. longa and R3, like as control + 2.30 % of C. longa. According to our results, feed intake did not differ among treatments (125 ± 13 g/d; $p = 0.32$) throughout the experiment. However, C. longa added at 1.30% (R2) had hicher final body weight ($p < 0.001$) whereas, R3 had higher feed conversion ratio ($p < 0.001$). Consequently, a statistic tendency on carcass yield was observed ($p = 0.08$). The R2 (77%) was higher than those observed by R1 (72%; $p = 0.03$) and T3 (73%; $p = 0.04$), being also huge this difference when compared to the Control treatment (63%; $p = 0.001$). In addition, serum lipid concentration was lower in R2 treatment than obtained for R1, R3 and Control ($p = 0.001$ to 0.023). Therefore, C. longa as a feed additive for animal production appears to be a valuable alternative instead of antibiotics, reducing also the products obtained with high carbon footprint. In the same way, we concluded that the use of C. longa flour at 1.30% as feed additive positively regulates lipid serum concentrations of Guinea pigs. These promising results, allow a wide, new range of further investigations at immunological levels for supporting the bioactive properties of C. longa.

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INTRODUCTION

The guinea pig or cavy *(Cavia porcellus)* is a domesticated rodent in the highlands of Peru, Ecuador, Colombia, and Bolivia for a period of at least 7000 years [1]. Its meat, similar in appearance and taste to rabbit or chicken meat, has been consumed for centuries by indigenous people [2]. Additionally, in South America the Cavia porcellus provides a significant amount of protein for rural smallholders, being part of their food security, contributing also in the reduction of malnutrition [3]. In Ecuador, Cavia porcellus is widely used in the Andean region by traditional-family breeding system, although with low technological levels. Furthermore, a qualitative study focusing on smallholders showed that farmers generally use indigenous crops instead of antibiotics to grow animals for household food supply [4]. Regardless of this, Cavia porcellus has a high prolificacy as well as great adaptability to a wide range of housing and management options [5], representing an important role in the gastronomical culture in the context of rural smallholders in this region.

In animal production, the use of antibiotics as a feed additive for controlling and preventing disease and for promoting growth is common throughout the world [6–8]. According to Van-Boeckel et al. [9] in 2010, an estimated 63,151 tons of antibiotics were used in food animal production worldwide, although predictions for 2030 are expected to increase to nearly at 67% (105,596 tons). Several scientific evidences have reported that, when bacteria, viruses, fungi and parasites no longer respond to medicines effectively is called an antimicrobial resistance (AMR) [10]. Therefore, AMR has global consequences for human health, resulting in approximately 700,000 deaths each year [11]. According to O'Neill [12] by 2050, it is projected that the number of AMR-related deaths could rise to 10 million annually, with an estimated economic impact of $100 trillion USD. In this sense, the World Organization for Animal Health [13] developed a list of restricted antibiotics for veterinary medicine and the European Medicines Agency (EMA) updated the categorization of antibiotics used in animals to promote prudent use [14]. All these guidelines seek to reduce AMR and to preserve the effectiveness of antibiotics for both humans and other animals.

In front of fight to AMR, social inequality and climate change, the UNO [15] proposed 17 sustainable development goals (SDGs) as an integral part of the 2030 agenda for sustainable development. Consequently, the SDGs mark a historic shift towards one sustainable development agenda after a long history of trying to integrate economic and social development with environmental sustainability [16]. Although all SDGs are related with animal production, the SDG 12 that focus to ensure sustainable consumption as well as SDG 3 of which objective is ensure healthy lives and promoting well-being for all at all ages, are the more directly involved [17]. In parallel, it is fundamental to mention that consumers of animal products are demanding for drug residues free meat. Therefore, a severe attention has been given to local food resources due to their bioactive compounds that in small quantities are able to modulate organic responses [18].

Based on the aforementioned scenario, natural growth promoters like plant extracts, could be used for feeding in Cavia porcellus without any adverse effect. Curcuma longa is widely used as a spice, colouring agent and is also known for its medicinal properties [19]. It is a rhizomatous perennial herb with a height of about 1.5 m, encountered in tropical and subtropical regions of the world [18]. The main active components of the rhizome are the non-volatile curcuminoids and the volatile oil [19]. Curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) are nontoxic polyphenolic derivatives of curcumin that exert a wide range of biological activities and pharmacological properties [21]. Despite that, Curcuma longa is also being used in animals such as rabbits [21,22], poultry [7,23–26] and rats [27,28]. There is a strong lack of research using Cavia porcellus to explore performance, metabolic and immunological responses. In the context of Ecuadorian conditions, despite Curcuma long is a local food usually used by rural smallholders, only few studies have been realized in order to understand about the inclusion of this local food resource with high antioxidant properties to be used as an alternative in the use of antibiotics as growth promoters.

Based on this, the current study aims to assess the inclusion of different Curcuma longa levels and their productive and lipid serum concentration, using *Cavia porcellus* as experimental model.

Materials and Methods

Ethical issues

All animal care, housing, and feeding procedures were adapted based on the World Organisation for Animal Health 2016 (animal welfare) and the current Ecuadorian regulations Organic Law on Animal Health No. 56, published in the Official Gazette, Supplement 27, 03 July 2017). However, this study did not require approval by an Institutional Animal Care and Use Committee because it did not involve animals used for scientific purposes as usually required by Directive 2010/63/EU (European Union, 2010) [Art. 2.5), This Directive shall not apply to the following:... (f) as practices not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.]

Experimental site

The present study was performed in the Orellana Province located in northeastern Ecuador, within the westernmost Amazonian lowland. The climate in this region is characterized by humid tropical rainforest conditions [29]. The average annual rainfall is of about 2942 mm with an average annual temperature of 29.7 °C, and altitudes of around 275 m above sea level.

Processing of Curcuma longa flour

For the experiment applied in the current study, the Curcuma longa was harvested in the town of Guayusa (Orellana province) and sundried for seven days. Thereafter, it was ground into powder using a hammer mill of sieve size 2 mm. The chemical analysis of Curcuma longa is listed in Table 1

Table 1. Phytochemical composition of Curcuma longa flour (values are means + standard deviation of three determinations

Phytochemicals	Composition (%)
Alkaloid	0.86 ± 0.01
Saponin	0.39 ± 0.01
tannin	1.10 ± 0.04
Sterol	0.04 ± 0.02
Hydrogen cyanide	0.84 ± 0.03
Flavenoid	0.38 ± 0.04
Phenol	0.07 ± 0.10

Animal Equipment and Housing

Some 120 animals, exclusively of males gender with an average of body weight 356 ± 25 g were used and housed in individual cages, which provided of drinkers and feeders. The cages were composed of metal mesh measuring 62 × 50 × 37.5 cm. In addition, throughout the experiment, controlled environmental conditions (20 to 25°C; 16HL:8HO) were maintained.

Animal Experimental Diet

A total of 120 Cavia porcellus of three weeks of age were allocated into four treatments in a completely randomized design (Control, R1, R2 and R3 of Curcuma longa flour). At the first step, all animals were subjected to adaptation period (seven days) with a basal diet (Control) consisting of Pennisetum purpureum and concentrate (60:40). The experiment lasted twelve weeks. Therefore, after adaptation, three experimental diets

were formulated by adding Curcuma longa flour, as listed in Table 2.

Table 2. Composition of experimental diets

Ingredients, %	Experimental diets			
	Control	R1	R2	R3
Pennisetum purpureum	60.75	60.75	60.75	60.30
Ground yellow corn	11	11	10.5	10.0
Rice powder	8.0	8.0	7.8	7.8
Palma oil	0.50	0.5	0.5	0.5
Cane molasses	0.50	0.50	0.50	0.50
Soybean meal	18.75	18.15	18.15	18.10
Vit . and Min. premix1	0.50	0.50	0.5	0.5
Curcuma longa flour	0	0.60	1.30	2.30
Total	100	100	100	100
Calculated composition				
Digestible energy, Mcal/kg	2.4	2.3	2.2	2.2
NDF, %	35	33	32	32
Crude protein, %	16	16	15	15

1 1Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D3,9790 IU; vitamin E, 121 IU; B 12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulphate, 60 mg; manganese oxide, 60 mg.

Measurement data

Performance responses and economic profit

Feed intake was daily recorded through a balance, capacity 2000 g ± 0.5 precision (GRAM FC, Madrid, Spain) whereas individual body weight (BW) was weekly recorded (GRAM FC, Madrid, Spain). The feed conversion ratios were calculated utilizing the data on body weight gain and feed intake.

Feed conversion ratio. FCR = feed intake (g)/weight gain (g)

In contrast, for carcass yields, after Cavia porcellus were slaughtered, bloodless and scalded (60–70°C during 45–60 seconds). This comprised the body after removal the hair, the head (between the occipital bone and first cervical vertebra), the hand and the feet at carpo-metacarpal and tarso-metatarsal joints, respectively, and the viscera [2]. The carcass retains the skin, lateral portions of the diaphragm and the perirenal and pelvic fat deposits. Finally, the economic profit was obtained between production costs and income.

Serum lipid concentration

For serum lipid concentration, five animals were randomly selected in each treatment. Blood samples were collected from ear vein in BD vacutainer (4 mL) without anticoagulant (BD Diagnostics, Franklin Lakes, NJ, USA) at one week prior to applicated treatments and at four, six, eight and twelve weeks after Curcuma longa supplementation before the morning feeding. The blood samples were allowed to rest during 24 h for the obtained serum and were stored at −20°C in 1.5 mL Eppendorf tubes until analysis. Then, through spectrophotometry method, HDL cholesterol and triglyceride were analysed as prescribed by the commercial kits.

Statistical analysis

All statistical analysis were performed using the SAS version 9.1.3 (SAS Institute Inc., Cary, NC). Previously, all data were checked with a normality test (PROC UNIVARIATE). Thereafter, productive data were subjected to one-way analysis of variance (ANOVA) through general linear model (GLM). In contrast, the metabolic responses were treated by repeated measure PROC MIXED of SAS. Regression analyses were obtained with the PROC REG procedure. Differences between least squares means were determined with the PDIFF test of SAS. Significance was declared at $P < 0.05$ unless otherwise indicated.

Results

Productive responses

The productive responses of *Cavia porcellus* subjected at different Curcuma longa levels, are listed in Table 3. No differences among treatments were observed when compared feed intake ($P = 0.32$: Table 3). The feed intake had a mean value of (124 ± 13 g/d, as indicated in Table 3). In the same way, initial BW did not differ when started experiment ($P = 0.60$) those averages were 355 ± 0.2 g of BW. Besides, previous to Curcuma longa levels administration, initial values of BW yielded a variation coefficient lower than 15%, which means that it remained homogeneous.

Table 1. Least square means of productive data when Cavia porcellus were subjected different Curcuma longa levels.

Items	Treatments[1]				SEM	*P* -Value
	Control	R1	R2	R3		Treatment
Feed intake, g/d	124	125	125	125	13	0.32
Initial body weight, g/d	356	357	353	354	0.2	0.60
Final body weight, g/d	498^d	595^c	698^a	644^b	1.7	0.001
Feed conversion ratio	3.6^a	3.1^b	2.7^c	2.4^d	0.10	0.001
Carcass yield, %	63^z	72^y	77^x	73^y	0.2	0.08

[1]Treatments; **Control**, same as adaptation; **R1**, Curcuma longa 0,60%; **R2**, Curcuma longa 1.30%; **R3,** Curcuma longa 2.30%; SEM, standard error of the means; $^{a-c}$ Means with different letter in the same row indicate statistics differences at $p < 0.05$; $^{x-z}$ Means with different letter in the same row indicate a statistics tendency at $P < 0.10$.

Final values of BW of *Cavia porcellus* after administration the different Curcuma longa levels, are listed in Table 3. The final values of BW differed when included Curcuma longa flour in diets of *Cavia porcellus* ($P < 0.001$). The R1 treatment, demonstrated hogher BW values (698 ± 1.7, on average) than those observed in R3 (644 ± 1.7, on average) and R1 (595 ± 1.7, on average), respectively, which also differed between them (Table 3). Whereas, when compared to those of Control treatment, huge statistic differences were detected (498 ±

1.7 g, on average; *P* = 0.03 a 0.001). In addition, the BW data presented a lineal tendency, although not statistically significant (*P* = 0.32; Figure 1).

As for feed conversion ratio, the R3 (2.4 ± 0.10%, on average; *P* < 0.001), R2 (2.7 ± 0.10%, on average; *P* < 0.002) and R1 (3.1 ± 0.10%, on average; *P* < 0.004) treatments had higher feed conversion than those observed in Control (3.6 ± 0.10%, on average; *P* < 0.001). Furthermore, the feed conversion ratio with regard to different Curcuma longa levels indicated an exponential adjustment (*P* = 0.04; Figure 2). In other words, the inclusion of 2.30% of Curcuma longa had highest feed conversion from a nutritional point of view. Consequently, it implies that there is no need of a high feed intake for achieving more BW.

Despite of clear numerical and statistical differences in the feed conversion among treatments (Table 3), the carcass yields only demonstrated statistical tendences (*P* = 0.08; Table 3). The inclusion of 1.30% of Curcuma longa had higher carcass yields (77 ± 0.13%; *P* < 0.001) compared to R1 (72 ± 0.84%) and R3 (73 ± 0.17%). It is fundamental to highlight that, all above results described differed than those to obtained for Control treatment (63 ± 0.11%; *P* < 0.001). However, the regression analysis resulted that the carcass yield had a polynomial adjustment that has been statistically significant (Figure 1; r = 0.98). Based to our results, this experiment appear to open a new study line, due to the properties of Curcuma longa as a potential feed additive to be included in the diets of Cavia porcellus eventually by small farmers.

As for economic terms, the cost production by each animal did not differ *P* < 0.42; USD 1.54) due to Curcuma longa has been obtained from the own farms. However, analyzing the economic profits, based on carcass yields, the current study demonstrated that the treatment R2 (1.30%; 5 USD/kg) had higher economic benefit than those obtained in Control (3 USD/kg) as well as for R1 and R3 which both obtained, on average 4 USD/kg.

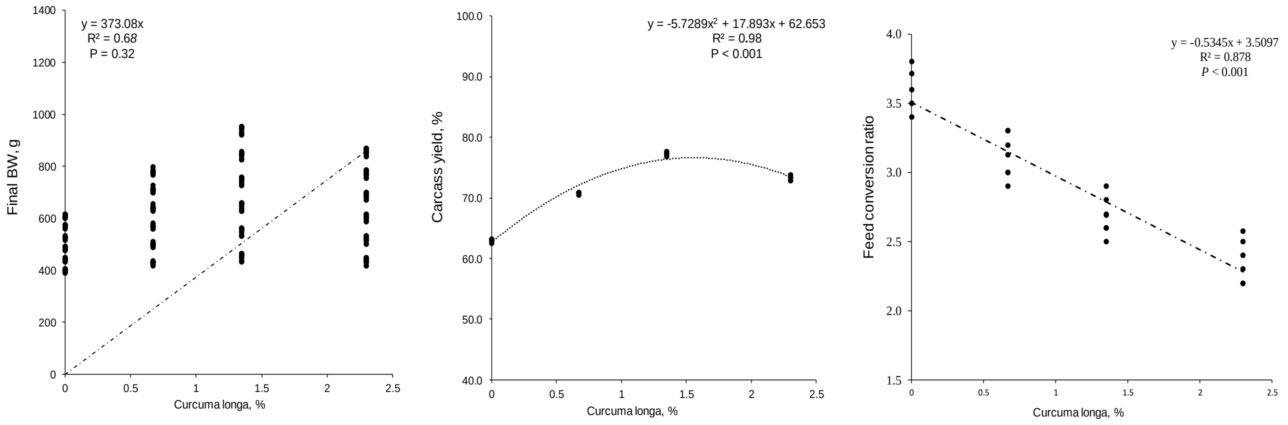

Figure 1. Regression adjustment for Final BW, Carcass yields and feed efficiency according to different Curcuma longa levels.

Concentration

Serum lipid concentration

Level effects of Curcuma longa flour incorporation on serum lipid concentration, are illustrated in Figure 2. One week prior to treatment applications, initial values of total cholesterol, HDL, LDL and triglycerides did not have statistic differences (P = 0.30 to 0.75). Nevertheless, serum concentrations of total cholesterol, HDL, LDL and triglycerides dramatically decreased (P < 0.05) with the inclusion of Curcuma longa flour in the diets over time (12 weeks). In addition, the highest relationships among HDL, LDL, triglycerides and Curcuma longa concentration yielded a high regression coefficient (r2 = 0.85 for HDL; r2 = 0.85 for LDL and r2 = 0.78 for triglycerides), which allows a strong relation between lipids concentration and the rate of inclusion Curcuma longa in feed. **eed.**

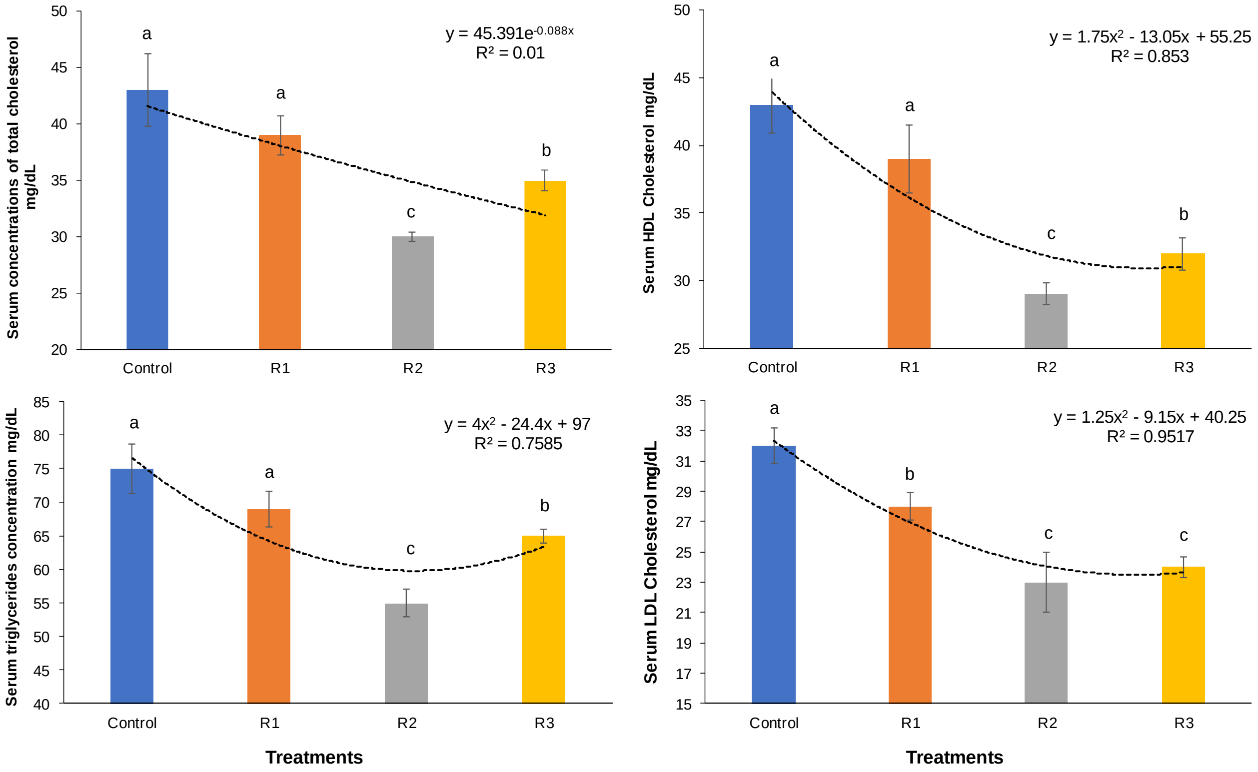

Figure 2. Effects of incorporation level of Curcuma longa flour on serum lipid concentration; Values represent an average of 12 weeks; a,b Means with different letter differ at $P < 0.05$. Treatments; Control, same as adaptation; R1, Curcuma longa 0,60%; R2, Curcuma longa 1.30%; R3, Curcuma longa 2.30%.

Discussion

Livestock production will increasingly be affected by external factors. These include surging demands for animal products and struggling supplies of feed raw materials, resulting from the competition for natural resources and trade barriers. At the same time, there is growing concern about food and its impact on health, and the impact of production systems on animal welfare and the environment [30]. In times of increasing worldwide occurrence of antibiotic-resistant bacteria, bioactive compounds (BC) are receiving considerable interest for its potential to efficiently destroy bacterial cells. Therefore, the BC represent an interesting food tool to include as functional foods in animal nutrition industry [31]. However, the effectiveness of a BC depends on the solubility, stability, and bioavailability.

In this sense, plants belonging to the genus Curcuma are gaining importance globally as one of the significant ingredients in food and traditional medicines [31]. Curcuma longa (syn. C. domestica Valeton and C. brog Valeton) is also known as “turmeric” worldwide, “kurkum” in Arabic, and “haldi” in Hindi and Urdu. Turmeric is cultivated extensively worldwide but is native to Southeast Asia [32]. An interesting study by Djoumessi-Tobou et al. [33] reported a DMI increase due to inclusion of Curcuma longa as a fed additive in *Cavia porcellus*. Besides, several animal models or human studies proved that curcumin is extremely safe even at very high doses [34]. However, Djoumessi-Tobou et al. [33] informed that high concentrations (> 1%, Curcuma longa, in DM) affected the DMI. Responses that in part could be explained due to the presence of alkaloids in the spices which could have induced a bitter taste to the feed. Whereas on the other hand, low solubility (due to their insolubility in water) and rapid metabolism would influence the bioavailability of curcuminoids [34,35] limiting also tissue distribution, apparent rapid metabolism and its short half-life. To support this, Shoba et al. [36] administered orally a dose of 2 g/kg of Curcuma longa in rats and reported a maximum serum concentration of 1.35 (0.23 µg/mL at time 0.83 h, whereas in humans the same dose of Curcuma longa resulted in either undetectable or extremely low (0.006 ± 0.005 µg/mL at 1 h) serum levels.

Despite the aforementioned evidence, in the present work when Curcuma longa was added at 1.30% in the diet in base to dry matter (DM), with the exception of DM intake (DMI), greater performance responses were observed than other proportions (0.60 and 2.3%). According to Al-yasiry et al. [37] in monogastric animals, the gastrointestinal mucosa is a line of defence against environmental pathogens. The objective is the fight against infectious and potentially harmful agents, a complex system of the submucosal and mucosal lymphatic tissue (GALT – gut- associated lymphoid tissue) has developed in the intestines. Consequently, curcumin as a fed additive included a modest concentration have could been partially absorbed in the intestine and also a considerable portion of the ingested curcumin reaches the cecum and colon, where a large population of indigenous bacteria exists [38]. Therefore, Curcuma longa has valuable properties such as antioxidant, free radical scavenging and modulating of immune and metabolic responses [7,20,39].

In the context of Ecuadorian conditions, due to *Cavia porcellus* are widely used for food security, being fed with mixed diet of forage, Curcuma longa might be an available feed additive for including in the diet limiting the application of antibiotic also contributing a less use of additives with high carbon footprint. As a result of the current research, we recommend this feeding strategy, although further studies should be performed at immunological level to support our findings.

Regarding the final body weight, supplying 1.30% of Curcuma longa had 11% higher body weight (698 g/d) than those obtained by Andrade-Yucailla et al. [40] when used at 3% (620 g/d). Whereas other studies in broiler and laying hen have reported higher body weight gain as well as feed conversion when they suplemented with 0.9% and 0.5% of Curcuma longa [24,26]. Although the Curcuma longa has widely been used as feed additive in animal production [37], limited studies have been made with *Cavia porcellus*. This perennial herb due to a wide variety of bioactive compounds has gained a lot of attention for including as a dietary strategy of supplementation by rural smallholders. Therefore, in our study providing 2.30% resulted to a higher feed conversion than obtained by Andrade-Yucailla et al. [37] and Djoumessi-Tobou et al. [37] when *Cavia porcellus* were supplemented with 3% of Curcuma longa. However, the carcass yield was a 7% greater in our study compared to those reported by Andrade-Yucailla et al. [37] (77 vs. 71 %). According to Djoumessi-Tobou et al. [33] the effects of curcuminoid may have favoured the secretion of emulsions at the level of the gall bladder, facilitating the digestion of nutrients, what possibly explains this greater feed efficiency.

On the other hand, hypercholesterolemia is one of the most important risk factors for atherosclerosis and subsequent cardiovascular diseases [28]. In this sense, several studies have been developed with animals as an experimental model trying to explain the association between risk of coronary heart disease and hypercholesterolemia [28,41]. In fact, our results, indicate that *Cavia porcellus* fed with Curcuma longa at 1.30% decreased the serum lipid concentration, due to increase of the activity of the enzyme cholesterol-7α-hydroxylase such as was observed by Hussein et al. [28]. Supporting these evidences, other studies in rabbits and broilers by Taha et al. [22], Hussein [42] and Abd-EL-Latif et al. [43], respectively, have reported significant decreases of cholesterol, triglycerides and LDL cholesterol concentrations when they were fed with Curcuma longa in doses between (0.25 to 2%, on DM basis). Consequently, we could hypothesize that the hypocholesterolemic effect of Curcuma longa may be attributed to its stimulatory effect on hepatic cholesterol-7α-hydroxylase enzyme, an enzyme that regulates cholesterol catabolism [44]. Besides this, Murugan & Pari [45] observed that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), triglycerides and free fatty acid levels dramatically decreased when Curcuma longa was added as feed additive. Based on these highlights, we conjectured that Curcuma longa have remarkable antioxidant, metabolic and immunological activities that allows them to be ideal candidates for their use in animal nutrition as an alternative for replacing to antibiotics [19]. Finally, in economic terms, our study yielded that using Curcuma longa a dose no greater than 1.30% might obtain 40% higher profit than those reported when *Cavia porcellus* were only forage fed.

Conclusions

Curcuma longa flour incorporated in the diet at 1.30% significantly improved the performances also decreasing the serum lipid concentrations. Consequently, local foods resources can be considered as a sustainable alternative for the application in animal production. Furthermore, it will help to promote the reduction to the loss of dependency on products with high carbon footprint within a scenario in the fight of climate change.

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COMPETING INTERESTS

The authors have no competing interests to declare.

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