UNIVERSIDADE FEDERAL DA BAHIA – UFBA

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

EFFECT OF DIETARY PROTEIN AND ENERGY CONTENTS IN HEPATIC AND RENAL METABOLISM IN SHEEP

FERNANDO CORREIA CAIRO

SALVADOR - BAHIA

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal da Bahia in partial fulfillment of the requirements for the degree of Doctor Scientiae.

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SUMMARY

EFFECT OF DIETARY PROTEIN AND ENERGY CONTENTS IN HEPATICAND RENAL METABOLISM IN SHEEP

Abstract: This study aimed to evaluate the effects of different concentrate levels and increased crude protein content in the diet on blood markers of hepatic and renal health in growing lambs. Two experiments were conducted with 64 intact male Santa Inês lambs, four months old, with an initial average weight of 20 ± 1 kg. The lambs were housed in individual pens and fed ad libitum during a 57-day experimental period, preceded by a 14-day adaptation period. The experimental design was a randomized complete block design (RCBD). Two experiments were conducted simultaneously to obtain the data used in this work. In Experiment 1 (Exp1), four diets with different concentrate levels (400, 500, 600, and 700 g/kg of DM) were tested. In Experiment 2 (Exp2), four diets with different crude protein levels (120, 140, 160, and 180 g CP/kg ofDM) were evaluated. Blood samples were collected on the 54th day in both experimentsfor analysis of serum albumin, urea, cholesterol, creatinine, alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT). The results indicated that the increase in concentrate level, expressed as digestible organic matter intake (dOM), did not significantly affect the blood parameters analyzed (P>0.05). However, serum urea levels were above the reference range (17 – 43 mg/dL) in Exp1, while creatinine levels were below the reference range (1.2 – 1.9 mg/dL). In Exp2, the increase in crude protein intake (CP) ledto a significant linear increase in serum urea levels (P=0.0002), although no significant changes were observed for AP, albumin, AST, ALT, GGT, creatinine, and cholesterol (P>0.05). This study demonstrates that increasing the intake of DOM and CP beyondthe requirements of confined lambs raises serum urea levels without affecting liver health, considering a 57-day experimental period.

Key words: Nutrition, health, urea, ruminants

Resumo: Este estudo teve como objetivo avaliar os efeitos de diferentes níveis de concentrado e o aumento do teor de proteína bruta na dieta sobre os marcadores sanguíneos da saúde hepática e renal em cordeiros em crescimento. Foram conduzidos dois experimentos com 64 cordeiros machos não castrados da raça Santa Inês, com quatro meses de idade e peso inicial médio de 20 ± 1 kg. Os cordeiros foram alojados em baias individuais e alimentados ad libitum durante um período experimental de 57 dias, precedido por um período de adaptação de 14 dias. O delineamento experimental foi em blocos casualizados (DBC). Dois experimentos foram realizados simultaneamente para obter os dados utilizados na elaboração deste trabalho No Experimento 1 (Exp1), foram testadas quatro dietas com diferentes níveis de concentrado (400, 500, 600 e 700 g/kg de MS). No Experimento 2 (Exp2), foram avaliadas quatro dietas com diferentes teores de proteína bruta (120, 140, 160 e 180 g PB/kg de MS). Amostras de sangue foram coletadas no 54º dia em ambos os experimentos para análise de albumina sérica, ureia, colesterol, creatinina, fosfatase alcalina (FA), alanina aminotransferase (ALT), aspartato aminotransferase (AST) egama-glutamiltransferase (GGT). Os resultados indicaram que o aumento no nível de concentrado expresso em consumo de matéria orgânica digestível (dMO) não afetou significativamente os parâmetros sanguíneos analisados (P>0,05). No entanto, os níveis de ureia sérica estavam acima da faixa de referência (17 – 43 mg/dL) no Exp1, enquantoos níveis de creatinina estavam abaixo da faixa de referência (1.2 – 1.9 mg/dL). No Exp2, o aumento da ingestão de proteína bruta (CP) levou a um aumento linear significativo nos níveis de ureia sérica (P=0.0002), embora não tenham sido observadas mudanças significativas para FA, albumina, AST, ALT, GGT, creatinina e colesterol (P>0,05). Este estudo demonstra que o aumento no consumo de dOM e CP acima das necessidades de cordeiros confinados elevam os níveis de ureia no soro sanguíneo, sem

afetar a saúde hepática considerando 57 dias de experimento.

Palavras chave: Nutrição, saúde, ureia, ruminantes

1. INTRODUCTION

Nutrition is fundamental for the efficient and sustainable production of ruminants, ensuring growth, performance and well-being. Proper formulation of diets, balancing protein, energy and the proportion of concentrate and forage is critical, as any mismatch can have adverse consequences for the animal and for the production system.

The biochemical profile is a valuable tool for nutritional and metabolic assessment in production animals (Schultz et al., 2022). Many metabolic markers can beused to evaluate nutrient adequacy in a diet. For accurate interpretation, it is essential to consider factors such as species, breed, climate, and physiological state (Onasanya et al., 2015). Dietary changes can overload the liver and kidneys, reflecting in alterations in blood markers of hepatic and renal health (Zang et al., 2017).

The metabolic profile and complete blood count (CBC) are important tools for monitoring metabolic adaptation, identifying nutritional imbalances, and aiding in disease prevention (Russel, 1991). These tests are particularly relevant in animals subjected to more intensive production systems, such as feedlots, where increased nutritional intake promotes rapid weight gain and shortens the production cycle through diets with higher proportions of concentrate. Enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase (AP) are markers of hepatic injury (Xue et al., 2021), and their activity can indicate the location and degree of cellular damage (González and Silva,
2022).

Studies show that diets with high levels of protein and concentrates can increase blood levels of urea and other metabolites, indicating an additional effort by the body to metabolize these nutrients (Wang et al., 2020a, 2020b). However, in many cases, increased protein intake does not result in greater utilization of this protein by the body (Wang et al., 2021). Diets rich in concentrates, with high intake of non-fibrous carbohydrates, can cause metabolic and digestive disturbances, such as acidosis, in addition to overloading the liver and kidneys (Ahmed et al., 2022).

The structure and function of the liver underscore the critical importance of eliminating ammonia, which is potentially toxic to ruminants. Enzymes involved in the ornithine cycle and transamination reactions are strategically located in the mitochondria and cytosol of periportal and perivenous hepatic cells. This arrangement allows for the conversion of ammonia, absorbed in the intestine, into urea, and also enables the use of glutamine synthesis as an additional mechanism to remove almost all the ammonia present in the hepatic portal blood (Meijer et al., 1990; Katz, 1992).

We hypothesized that high concentrate ratios and a crude protein content above the nutritional requirements in the diet can alter blood markers of hepatic and renal health, above and below the reference values, in growing lambs. Then, the objective of this work was to evaluate the effect of different concentrate ratios and increasing dietaryprotein content on the blood markers of hepatic and renal health in growing lambs.

2. MATERIALS AND METHODS

2.1. Ethical considerations and location of experiments

Two experiments were carried out to obtain the data to prepare this work. Both experiments were conducted following the principles of ethics and animal welfare and

were approved by the Ethics Committee on Animal handling and care of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (CEUA – UFBA), under protocol numbers 34/2020 and 36/2020.

The experiments were carried out at the experimental farm of the Federal University of Bahia (Universidade Federal da Bahia – UFBA), located in the municipality of São Gonçalo dos Campos, Bahia State, Brazil. The chemical analyses ofthe collected samples were analyzed in the Laboratory of Animal Nutrition of the same institution.

2.2 Animals, experimental design, and diets

A total of 64 male Santa Inês lambs, non-castrated, with an initial age of four months and an average initial weight of 20 ± 1 kg, were used. The animals were housed in individual pens (1.2 m^2), covered, with suspended and slatted floors provided with individual feeders and drinkers. Animals were fed ad libitum and received free access to water throughout the experimental period. In the first experiment (Exp1), a total of 32 animals were distributed in a randomized block design with four treatments. The four groups of eight animals received each one of the four experimental diets (Table 1), which were increasing concentrate contents 400, 500, 600, and 700g of concentrate/kgof DM). The forage contents were decreased proportionally for all treatments.

Both experiments were carried out simultaneously at the same time.

The two experiments were derived from two performance and meat quality experiments that were carried out simultaneously. The lambs were kept in feedlot for 57 days, preceded by a 14 days of adaptation period, during which the animals were adapted to the location and experimental diets. During the adaptation period, the animals were

identified, subjected to endo- and ectoparasites control, and immunized with polyvalent vaccines against rabies and clostridial disease.

In the second experiment (Exp 2), another 32 animals were also randomly distributed among four groups of eight animal in a randomized block design. Experimental treatments were four diets formulated with increasing crude protein (CP) contents (Table 1), which were 120, 140, 160, 180 g CP/kg DM. The diet 160 g CP/kg DM was formulated to meet the requirements of hair sheep raised in tropical conditions, with an estimated average daily gain (ADG) of 250 g/day, in accordance with NRC (2007). Other two diets were formulated with CP below and one with CP above the stablished requirement. The diets in Exp 1 were formulated to be isonitrogenous (160 g/kg of crude protein) and to meet the nutritional requirements for lambs with an estimated potential average weight gain of 250 g/day.

The both diets consisted of corn silage as a the forage source. The concentrate was formulated using soybean meal, ground corn, urea, mineral mixture, limestone, and sodium bicarbonate.

2.3 Experimental Procedures and Sample Collection

The diets were distributed twice daily, at 09h00 and 16h00, in equivalent portions, presented as a total mixed ration, allowing for a leftover of 10 to 15% based on the offered fresh feed. After 24 hours, leftovers were collected, weighed, andadjusted to promote the *ad libtum* intake, and to record the dry matter (DM) and nutrients intake individually by animal. To determine the intake of each nutritional component, individual refusals were collected daily before the morning feeding and subsequently weighed on a digital scale. The feed offered and refusals were sampled weekly during the experimental period. These samples were then stored in plastic bags, identified, and kept in a freezer

at -20ºC. Thus, nutrient intake was estimated by the difference between the total amount of each nutrient in the offered feed and the amount in the refusals for DM, neutral detergent fiber corrected for ash and protein (apNDF), organic matter (OM), crude protein (CP), ether extract (EE), total carbohydrates (TC), and non fibrous carbohydrates (NFC)

The digestibility trial was conducted on days 44-46 and 55-57 of the experimental period of each experiment, totaling three consecutive days of fecal collection, with two spot samples collected per day at 8:00, 10:00, 12:00, 14:00, 16:00, and 18:00 hours (Lazzarini et al. 2016). Immediately after collection, the fecal samples were pre-dried in a forced-air oven (55ºC for 72 hours) and then ground in a knife mill (Willey mill; TECNAL, São Paulo, SP, Brazil) equipped with 1 and 2-mm sieves. After grinding, composite samples were prepared proportionally based on dry weight for each animal per period, packed in identified plastic bags, and stored at room temperature.

To estimate fecal output, indigestible neutral detergent fiber (iNDF) was used as an internal marker. For this analysis, approximately 0.5 g of refusal, feces, and diet ingredient samples were weighed in triplicate and placed in non-woven fabric bags (TNT) (100 g/m2, 50 μm; 4 x 5 cm) at a ratio of 20 mg of air-dried sample per cm² of surface area The fistulated cattle were adapted to a basal diet and bags were *in situ* incubated during 288 hours (Reis et al., 2017). All bags were removed from the rumen, manually washed until the water was clear, and then partially dried in a forced-air oven (55ºC for 72 hours). Subsequently, the bags were treated with a neutral detergent solution and maintained in an autoclave at 120°C for 60 minutes, washed in boiling water and acetone. They were then pre-dried in a forced-air oven (55ºC for 72 hours) and placed in a non-ventilated oven at 105ºC for 45 minutes for total drying. After, bags
were destinated to NDF analysis to quantify the iNDF. Blood samples were collected on the 54th day of each experiment, four hours after the morning feeding, a few days before the

end of the experimental period. The animals were weighed at the end of the experiment, after fasting, on the 57th day, with the following weights: Exp1 36.54 ± 12.3 kg and Exp2 29.1 ± 11.4 kg. Approximately 10 ml of blood were collected individually in a sterile test tube with a clot activator from all animals. The samples were temporarily kept at room temperature until clot retraction occurred and then centrifuged at up to 1370 RCF for 15 minutes to obtain serum. Finally, the serum was stored at -20°C until analysis.

2.4 Laboratory Analyses

Chemical analyses were carried out at the Animal Nutrition Laboratory, belonging to the School of Veterinary Medicine and Animal Science at the Federal University of Bahia. Samples of ingredients, refusals, and feces were analyzed according to the protocols described by the Brazilian National Institute of Science and Technology in Animal Science (INCT CA; Detmann et al., 2021). The following method numbers were used: DM (method G 003/1), ash (method M 001/2), EE (method G 005/2), apNDF (method F 002/2).

The total nitrogen (N) content in the samples of offered ingredients, refusals, and feces was measured by the Kjeldahl method, and CP was calculated as N × 6.25 (INCT-CA; method N-001/2, Detmann et al., 2021). The OMcontent of forage and feeds was determined using ash values.

The NFC content of the diets was calculated according to the equation proposed by Hall (2000): NFC = 100 – [(dietary CP % - urea CP % + dietary urea %) + apNDF % + EE % + ash %]. Total carbohydrates (TC) were estimated using the equation proposed by Sniffen et al. (1992): TC = 100 - (CP % + EE % + ash %).

The iNDF provided from the TNT bags were analysed according to INCT CA-F 009/1 method and calculated using the equation proposed by Detmann et al. (2012).

Serum samples were analyzed for total albumin (ALB), urea, total cholesterol, triglycerides, creatinine, alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) concentrations using commercial kits (Labtest Diagnostica). Biochemical analyses were processed using a semi-automatic device (Bioplus 2000®). The device was previously calibrated with Calibra 1 in conjunction with Qualitrol 1 H universal control serum (Labtest Diagnostica). The reference values used in the study were proposed by González and Silva (2022) and the hepatic enzymes by Kaneko et al. (2008).

The glomerular filtration rate (GFR) was calculated according to Cockcroft(1976) using the formula: GFR=(UCr * V)/PCr, where: GFR= Glomerular Filtration Rate (ml/min), UCr= Urine creatinine concentration (mg/dL), V= Collected urine volume per minute (ml/min), PCr= Plasma or serum creatinine concentration (mg/dL).

2.5. Statistical Analysis

The data from both experiments were analyzed using the MIXED procedure of SAS (OnDemand for Academics version). The treatments applied (protein levels or concentrate levels) were considered fixed effects in the model, along with the effect of blocks. A random regression model was evaluated considering each blood metabolite as a dependent variable and the protein or dOM intake per kg of BW as an independent variable. In this context, the slope and intercept of the model were considered random variables to verify if there is a linear relationship between the variables. The estimates of the intercept and slope were considered significant or not using 0.05 as the critical

probability level for type I error.

3. RESULTS

In Exp1, with the increase in concentrate in the diets, the average DM intake was 1029.2 g/day, with a standard deviation of ±152.3 g, while the average DM digestibility was 73.1%. In Exp2, with the increase in CP in the diets, the average DM intake was 1335.9 ± 204.7 g/day, with an average digestibility of 69.8%. It was observed that OM and CP intake and digestibility followed similar trends, with higher average values in Exp2 (Table 2).

No significant changes were observed in the evaluated blood parameters (P>0.05) (Figures 1 and 2). The levels of the enzymes AP, AST, ALT, GGT, albumin, and cholesterol remained within the ideal range, below the recommended limits for sheep, which are <387, 68-280, <32, 60-280 U/L, 2.6-4.2, and 52-76 mg/dL, respectively.

Urea levels remained above the ideal range (17-43 mg/dL) in Exp1 for animals that consumed a diet with an average of 16.8% CP, 884.5 g/kg BW of CP, between 434.9 and 1072.2 g/day of digestible organic matter, and an average intake of 172.9 g/day of CP. Creatinine values were below the ideal range (1.2-1.9 mg/dL), and cholesterol remained within the recommended range (52-76 mg/dL) (Figure 4). The increase in CP intake in Exp2 promoted a significant linear increase in serum urea levels (P=0.0002) (Figure 6). However, no statistically significant differences were observed for AP, albumin, AST, ALT, GGT, creatinine, and cholesterol (Figures 5 and 6) (P>0.05).

In Exp2, the results were similar to Exp1, with the levels of AP, AST, ALT, GGT, albumin, and cholesterol within the ideal range (Figures 7 and 8). Urea levels exceeded the reference values (RV: 17-43 mg/dL) when CP intake was above 200

g/day, with diets ranging from 11.64% to 20.15% CP, while creatinine (RV: 1.2-1.9 mg/dL) showed values below the reference range, as shown in (Figure 8). For the glomerular filtration rate (GFR), no changes were observed in both experiments (P>0.05), (Figure 9).

4. DISCUSSION

Blood metabolites are essential tools for verifying the adequacy of nutrient ;.,m/,bnbbb absorption, the functionality of vital organs, and the detection of any potential damage to the animal (Sa et al., 2014; Puppel and Kuczyńska, 2016; Ding et al., 2016). González and Silva (2022) estimated reference ranges for blood metabolites in sheep, including cholesterol (52–76 mg/dL), creatinine (1.2–1.9 mg/dL), urea (17–43 mg/dL), and albumin (26–42 g/L). For hepatic enzymes, the recommended values are aspartate aminotransferase (AST) (68–387 U/L), alanine aminotransferase (ALT) (<30 U/L), gamma-glutamyl transferase (GGT) (60–280 U/L), and alkaline phosphatase (AP) (<387 U/L) according to Kaneko et al. (2008).

The absence of differences in serum urea levels observed in Exp1 can be attributed to the diets being isoproteic. This suggests that variations in digestible organicmatter intake did not significantly influence protein utilization, although blood serum urea levels were above the ideal range for all animals as recommended by González andSilva (2022) (17–43 mg/dL). When urea and creatinine levels are within the normal range, it can be inferred that renal function was not compromised by the diets offered (Sauberlich et al., 1981; Payne and Payne, 1987; Vieira et al., 2008). However, despite elevated urea levels and low creatinine levels, the enzymes AP, AST, ALT, and GGT

remained within normal ranges, suggesting the absence of liver damage, even with the increase in circulating blood urea.

In Exp1, the animals consumed an average of 172.9 g/day of crude protein (CP), while the recommended intake according to the BR-Caprinos e Ovinos (2024) requirements table for sheep in the same category as our study is 149.75 g/day of CP. Studies indicate that high-protein diets can elevate blood urea levels, suggesting an additional effort by the liver to metabolize these nutrients (Wang et al., 2020a; Wang et al., 2020b). However, in many cases, increased protein intake does not result in greater utilization of this protein by the body (Wang et al., 2021). This suggests that the increase in blood serum urea levels reflects waste and a potential overload for the liver. Nevertheless, this overload did not increase the hepatic enzymes AP, AST, ALT, and GGT, nor the creatinine levels, demonstrating that the elevated protein intake did not cause liver damage.

In Exp2, with increasing levels of CP, the animals consumed an average of 215.1 g/day, with average values of 143.0, 201.8, 248.5, and 256.47 g/day of CP when separated by treatments, an average of 1335.9 g of dry matter (DM), and a weight of 29.1 ± 11.4 kg. According to the recommendations of the BR-Caprinos e Ovinos (2024), uncastrated sheep with a weight of 30 kg and a daily gain of 200 g require an intake of 1125 g of DM and 149.75 g/day of CP. These values help explain why blood urea levels remained within the ideal range for animals consuming CP in quantities below their requirements. However, when CP intake exceeded the recommended levels, urea levels increased, exceeding the recommended range of 17–43 mg/dL for sheep, as suggested by González and Silva (2022).

Wang et al., (2020), evaluating increasing protein levels in the diet (125, 132, 140, 148, and 156 g/kg of CP) of confined sheep with a forage ratio of 30:70,

demonstrated a significant linear increase (P=0.002) in the amount of nitrogen in the form of urea present in the blood (19.27, 22.52, 22.91, 24.93, and 23.58 mg/dL).

However, the values remained within the ideal range for sheep in all treatments. When evaluating the hepatic enzymes AST and ALT to investigate liver damage, the valuesfor all treatments were within the ideal range: AST (109.2 ± 7.2 U/L) and ALT (21.07 ± 2.0 U/L).

Enzymes such as ALT, AST, GGT, and AP are used as markers to determine liver damage, resulting in increased hepatocyte permeability (Stojević et al., 2005). Gonzalez and Silva (2022) showed that the activity of these enzymes allows inferences about the location and degree of cellular damage, as their increase is directly linked to liver damage from various sources—liver, skeletal muscle, heart, and kidneys. In our study, the enzymes AP, ALT, AST, and GGT did not increase with higher intake of dOM or CP and remained within the reference range proposed by Kaneko et al., (2008). This information helps to demonstrate that the excess circulating blood urea did not cause liver damage in confined sheep. These values are consistent with Magalhães et al.,(2021), who, in a study with 80 confined lambs with a forage ratio of 50:50 containing 171 g/kg of CP, found average values of 18.6 ± 0.6, 81.45 ± 0.2, and 54.1 ± 1.3 U/L for ALT, AST, and GGT, respectively.

Cholesterol levels suggest that there were likely no deficiencies in energy metabolism and that there was no excess of fat reserves being mobilized (Homem et al., 2010; Rodrigues et al., 2010). Magalhães et al., (2021) found cholesterol values ranging from 67.8 to 75.2 mg/dL, which are within the acceptable range for confined lambs according to Gonzales and Silva (2022). These values are consistent with those found in this study, both for the increased intake of dOM and CP, with respective averages of

65.09 and 62.7 mg/dL.

The glomerular filtration rate (GFR) is widely used to assess kidney function, even when not measured directly, and is commonly estimated through plasma and urinary creatinine clearance (Kamili et al., 2013). GFR can be influenced by dietary protein levels, such that a reduction in GFR may result in lower excretion of metabolites in the urine, including creatinine. To investigate potential changes in kidney function in response to diet, GFR is the most frequently employed laboratory test, serving as an important indicator of creatinine clearance (Kirsztajn, 2009; Manuwar et al., 2017).However, in both experiments analyzed, no significant differences in (GFR) were observed.

5. CONCLUSION

The increase in dOM intake does not affect the evaluated blood metabolites. The intake of 172.9 g/day of CP raised blood urea levels in confined sheep and reduced creatinine values. However, hepatic enzyme levels are within the recommended ideal range, indicating the absence of liver damage. The increase in protein intake raised serum urea levels when consumption exceeded the requirements of confined intact lambs; however, no hepatic damage was observed, as the FA, AST, ALT, and GGT enzymes remained within the ideal range for growing sheep, considering a 57 day confinement experimental period.

Table 1 - Proportion of ingredients and chemical composition of the experimental diets.

Item	Exp 1				Exp 2			
	Concentrate levels (g/kg DM)				Crude protein levels (g/kg DM)			
	400	500	600	700	120	140	160	180
Corn silage	600	500	400	300	500	500	500	500
Soybean meal	145	145	145	145	40	85	145	190
Ground corn	231	332	427	528	437	391	332	286
Urea/AS[1]	4	3	3	2	4	5	4	5
Mineral mix[2]	10	10	10	10	10	10	10	10
limestone	10	10	10	10	10	10	10	10
Sodium bicarbonate	0	0	5	5	-	-	-	-
Chemical composition								
Dry matter	525	583	641	699	579	581	583	584
Organic matter	939	941	939	942	965	962	959	957
Ether Extract	27	29	30	32	31	30	29	28
Crude protein	166	165	164	163	118	141	165	188
apNDF[3]	316	280	244	208	292	290	289	286
Non-fibrous carbohydrates	437	473	506	542	451	487	522	557
Total carbohydrates	753	753	750	751	802	779	751	729
TDN[4]	768	775	779	786	738	733	728	723

1 Urea/ammonium sulfate ratio of 9:1; 2 Provides per kg: calcium 110 g; phosphorus 87 g; sulfur 18 g; sodium 147 g; cobalt 15 mg; copper 590 mg; chromium 20 mg; iodine 50 mg; manganese 2000 mg; molybdenum 300 mg; selenium 20 mg; zinc 3800 mg; fluorine 870 mg. 3 Neutral detergente fiber corrected for ash and protein; TDN.

Table 2. Descriptive statistics of the intake and digestibility of of diets with increasing contents of concentrate (Exp 1) or crude protein (Exp 2).

Item	Exp 1					Exp 2				
	Concentrate levels					crude protein levels				
	Intake									
	Mean	Median	Minimum	Maximum	Standard deviation	Mean	Median	Minimum	Maximum	Standard deviation
Dry matter (g/d)	1029.2	1030.8	687.6	1503.8	152.3	1335.9	1337.2	930.1	1702.9	204.7
Organic matter (g/d)	992.1	987.6	663.6	1438.9	147.3	1253.8	1257.5	878.4	1596.3	192.0
Digestible organic matter (g/d)	739.4	736.6	434.9	1072.2	123.4	885.7	900.4	623.8	1136.1	161.6
dOM (g/kg BW)	20.3	20.4	13.02	31.54	2.6	44.3	43.3	28.33	70.2	7.3
Crude protein (g/d)	172.9	174.1	114.8	256.3	25.7	215.1	208.3	110.0	332.5	46.8
Crude protein (g/kg BW)	4.8	4.6	3.6	7.5	1.4	7.62	7.5	3.6	13.1	1.7
apNDF[1] (g/d)	293.6	297.1	190.3	510.6	47.6	334.39	338.9	240.4	429.3	53.7
	Digestibility (g/ kg DM)									
	Mean	Median	Minimum	Maximum	Standard deviation	Mean	Median	Minimum	Maximum	Standard deviation
Dry matter	731.3	732.0	648.2	806.4	28.7	698.2	701.0	593.4	816.5	40.3
Organic matter	745.7	74.7	666.6	820.2	28.2	711.2	713.6	623.0	830.4	37.4
Crude protein	701.8	727.3	535.1	785.0	46.7	652.0	652.8	518.8	805.3	54.8
apNDF[1]	579.2	581.7	440.7	720.1	56.7	368.8	352.6	234.0	573.1	76.1

1 Neutral detergente fiber corrected for ash and protein.

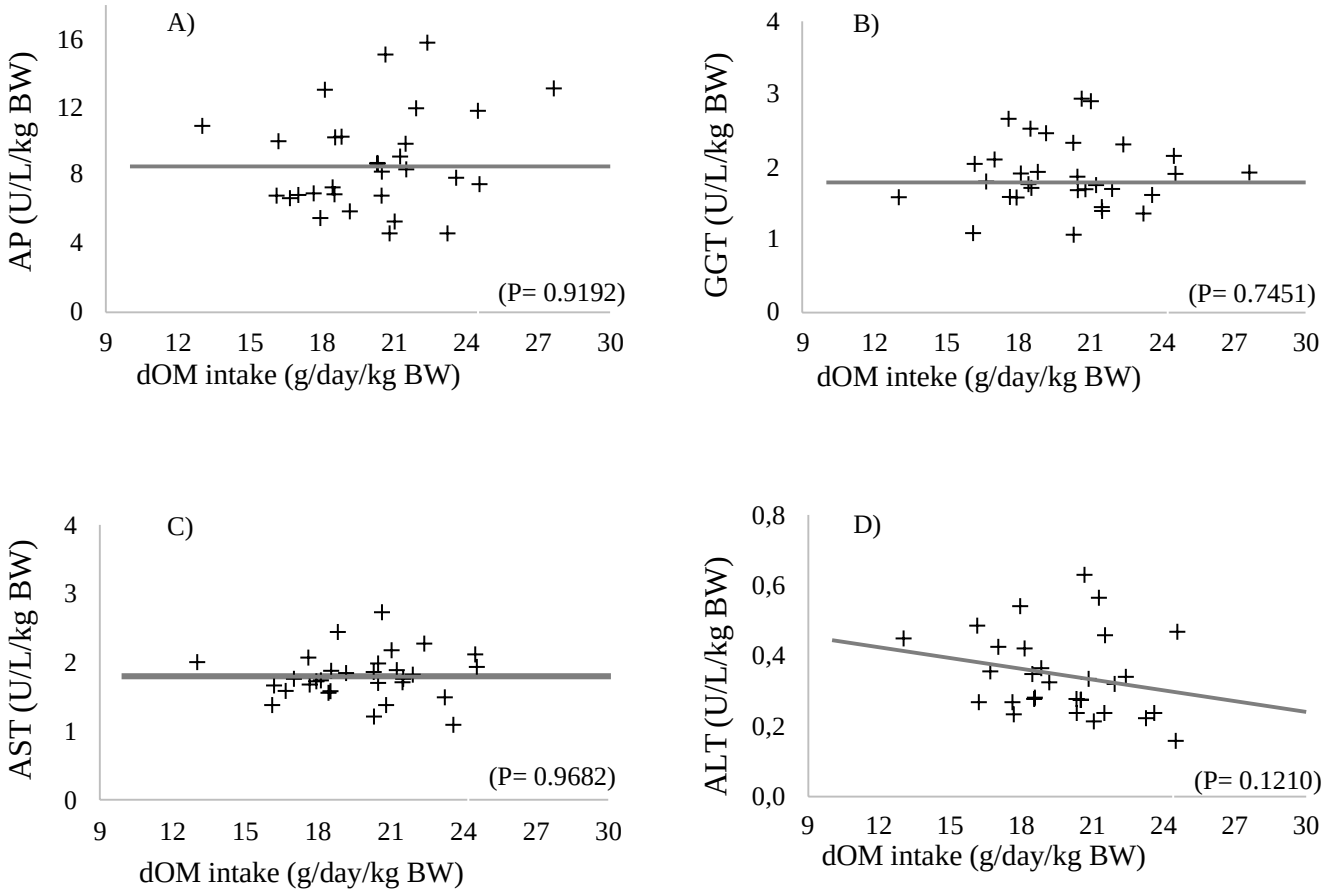

Figure 1. Relationship between intake of digestible organic matter (dOM) (g/day/kg BW) and the followed enzymes A) Alkaline phosphatase (AP), B) Gamma-glutamyl Transferase (GGT), C) Aspartate Aminotransferase (AST), and D) Alanine Aminotransferase (ALT) (U/L/kg BW) in the serum of sheep.

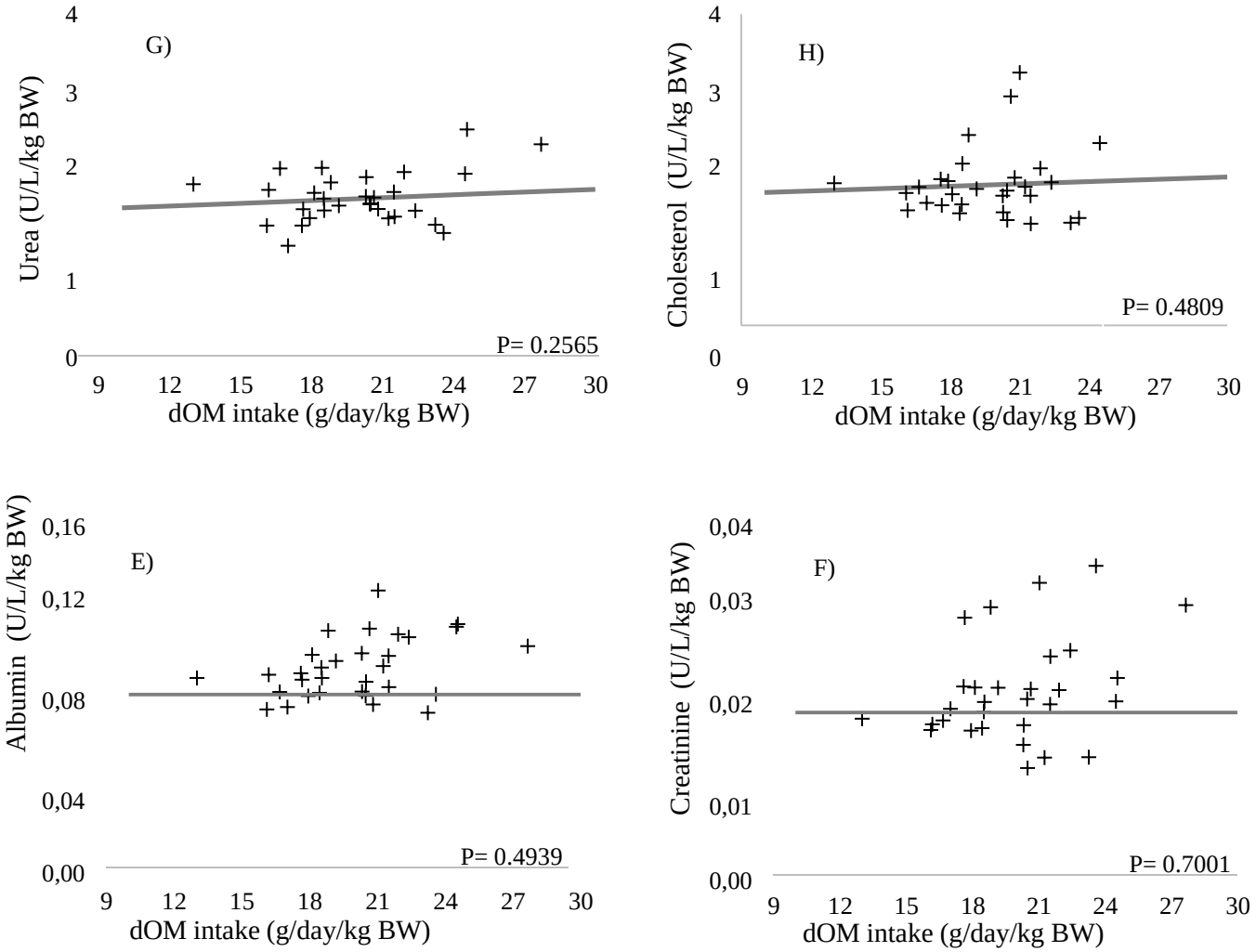

Figure 2. Relationship between intake of digestible organic matter (dOM) (g/day/kg BW) and the followed metabolites E) Albumin, F) Urea, G) Creatinine, and H) Cholesterol in the serum of sheep.

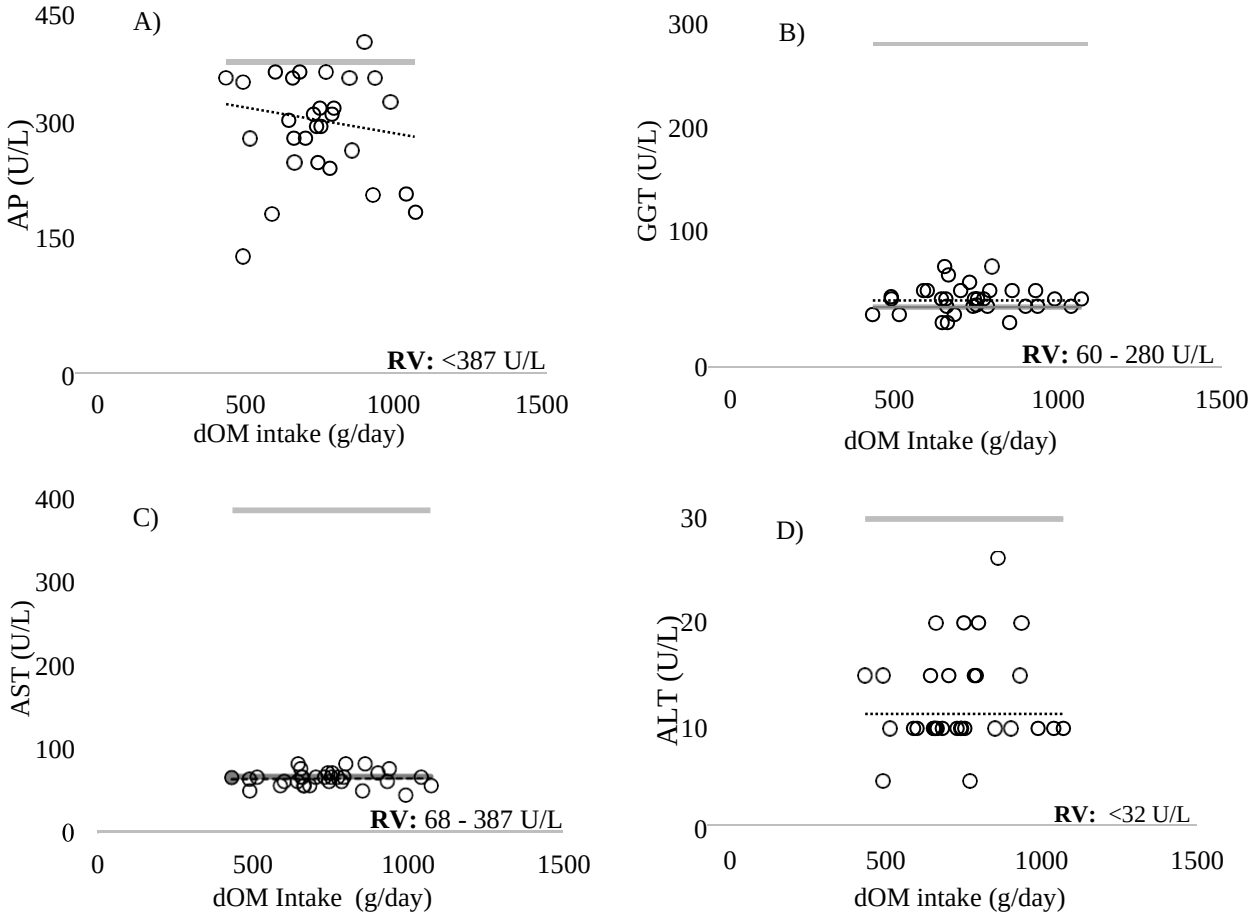

Figure 3. Relationship between intake of digestible organic (dOM) (g/day) and the values of enzymes. A) Alkaline Phosphatase (AP), B) Gamma-Glutamyl Transferase (GGT), C) Aspartate Aminotransferase (AST), and D) Alanine Aminotransferase (ALT) (U/L/kg BW) in the serum of sheep, the light gray line represents the maximum value and the dark gray line the minimum value according to the reference values RV: maximum (▬), minimum (▬) (Kaneko et al., 2022).

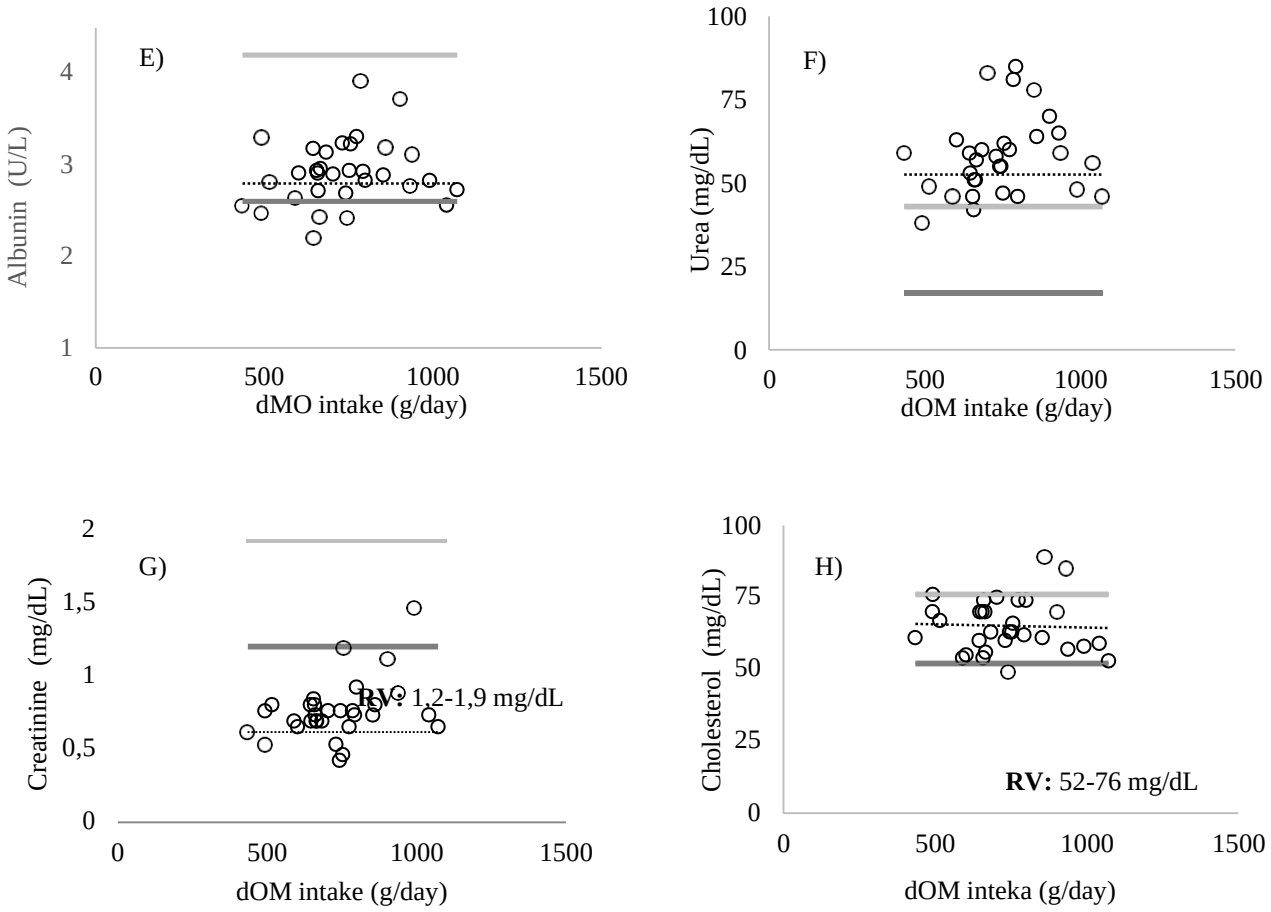

Figure 4. Relationship between intake of digestible organic (dOM) (g/day) and the values of the metabolites E) Albumin, F) Urea, G) Creatinine, and H) Cholesterol in the serum of sheep, the light gray line represents the maximum value and the dark gray line the minimum value according to the reference values RV: maximum (—), minimum(—) Gonzales and Silva (2022).

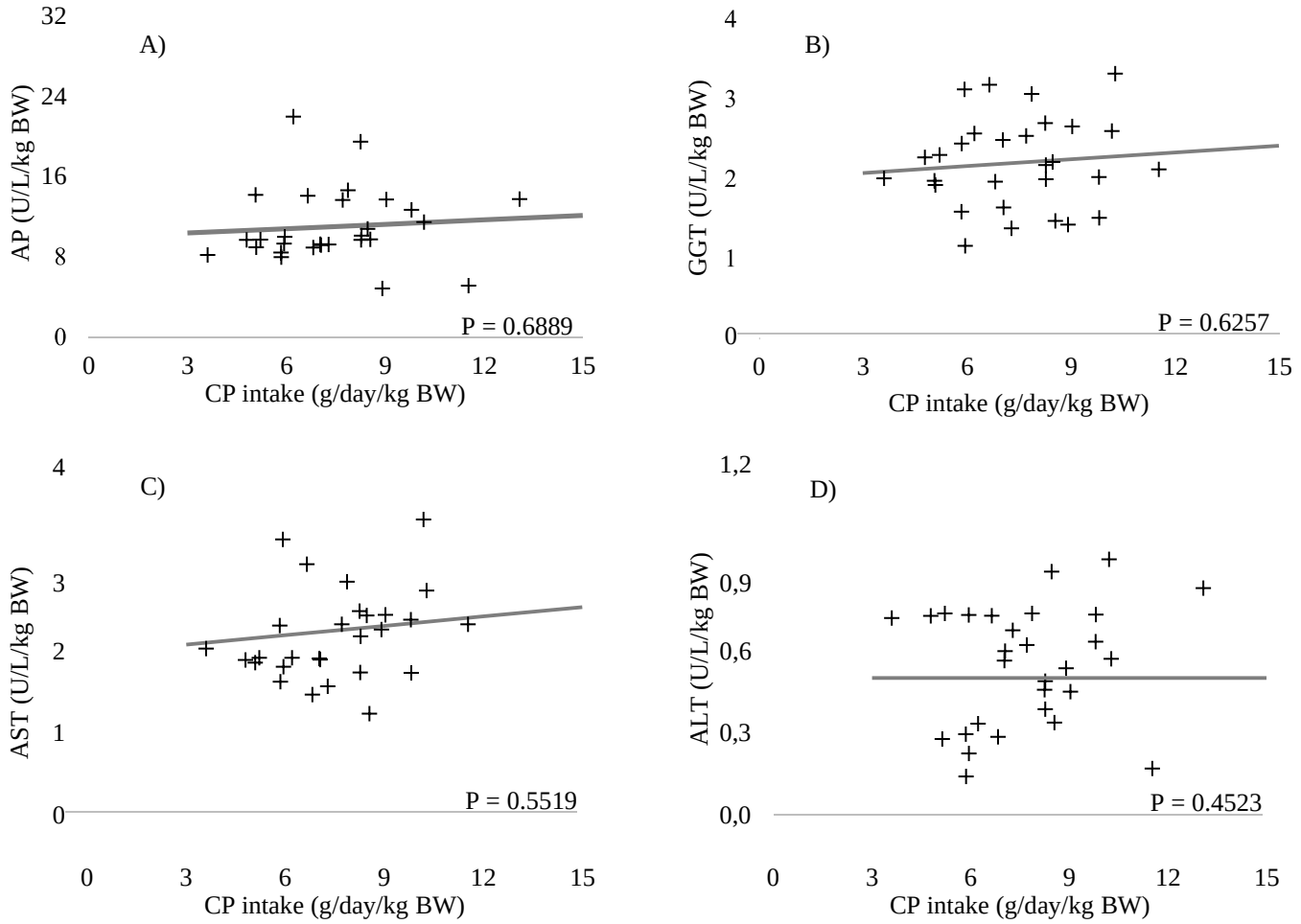

Figure 5. Relationship between crude protein (CP) intake (g/day/kg BW) and thefollowed enzymes A) Alkaline phosphatase (AP), B) Gamma-glutamyl Transferase (GGT), C) Aspartate Aminotransferase (AST), and D) Alanine Aminotransferase (ALT) (U/L/kg BW) in the serum of sheep.

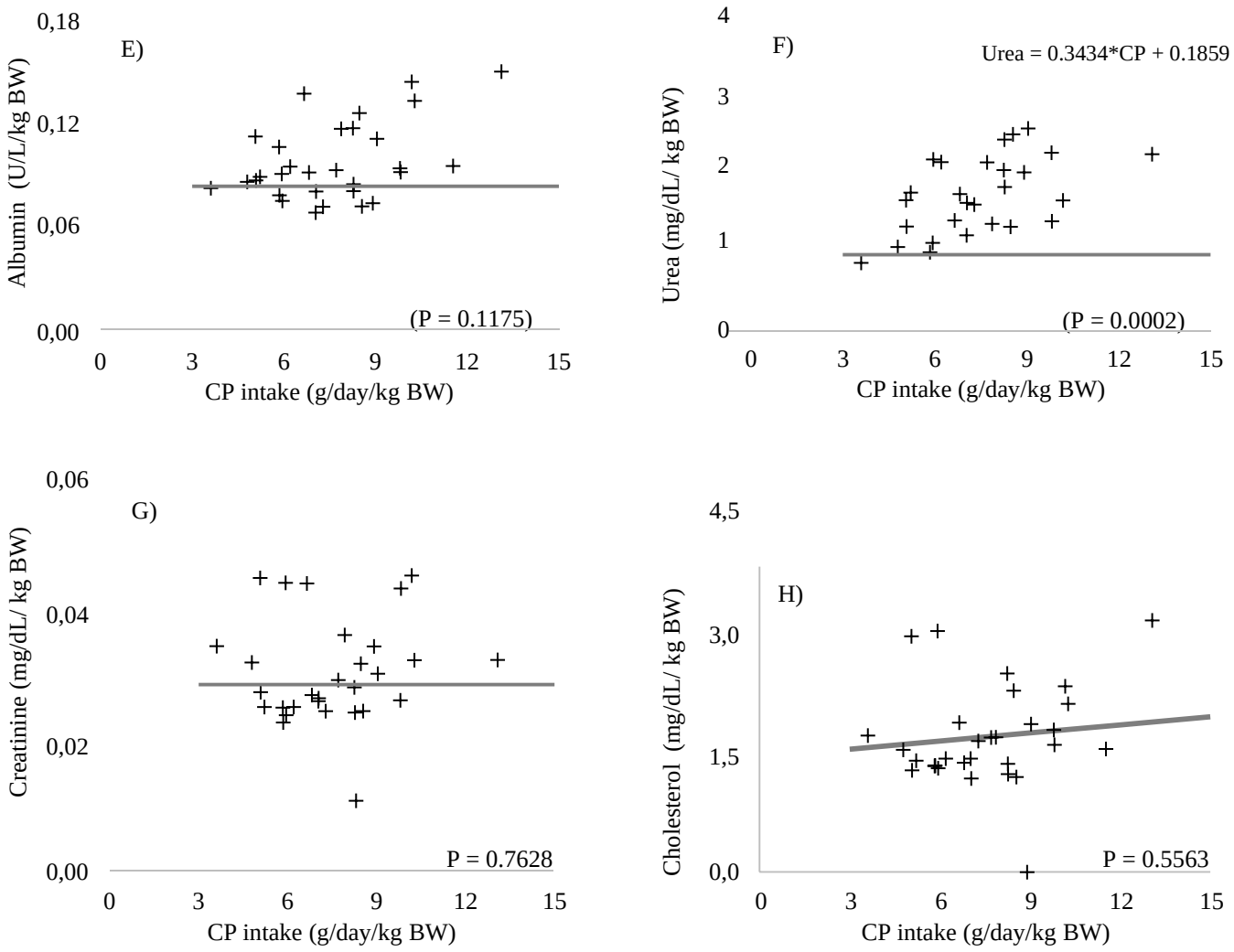

Figure 6. Relationship between crude protein (CP) consumption (g/day/kg BW) and the values of the metabolites E) Albumin, F) Urea, G) Creatinine, and H) Cholesterol in the serum of sheep.

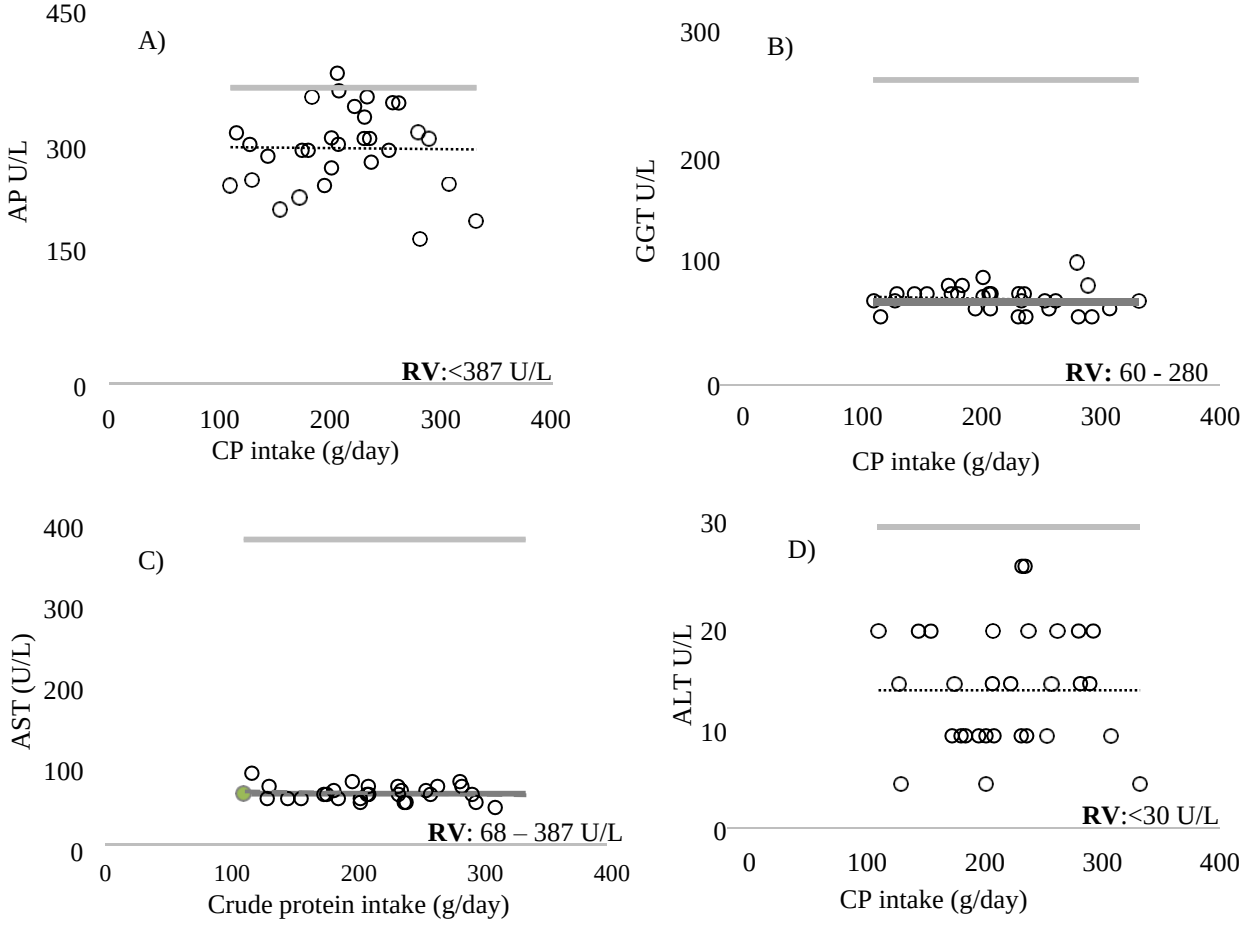

Figure 7. Relationship between crude protein (CP) intake (g/day) and the values of enzymes. A) Alkaline Phosphatase (AP), B) Gamma-Glutamyl Transferase (GGT), C) Aspartate Aminotransferase (AST), and D) Alanine Aminotransferase (ALT) (U/L/kg BW) in the serum of sheep the light gray line represents the maximum value and the dark gray line the minimum value according to the reference values RV: maximum( ), minimum ( ) (Kaneko et al., 2008).

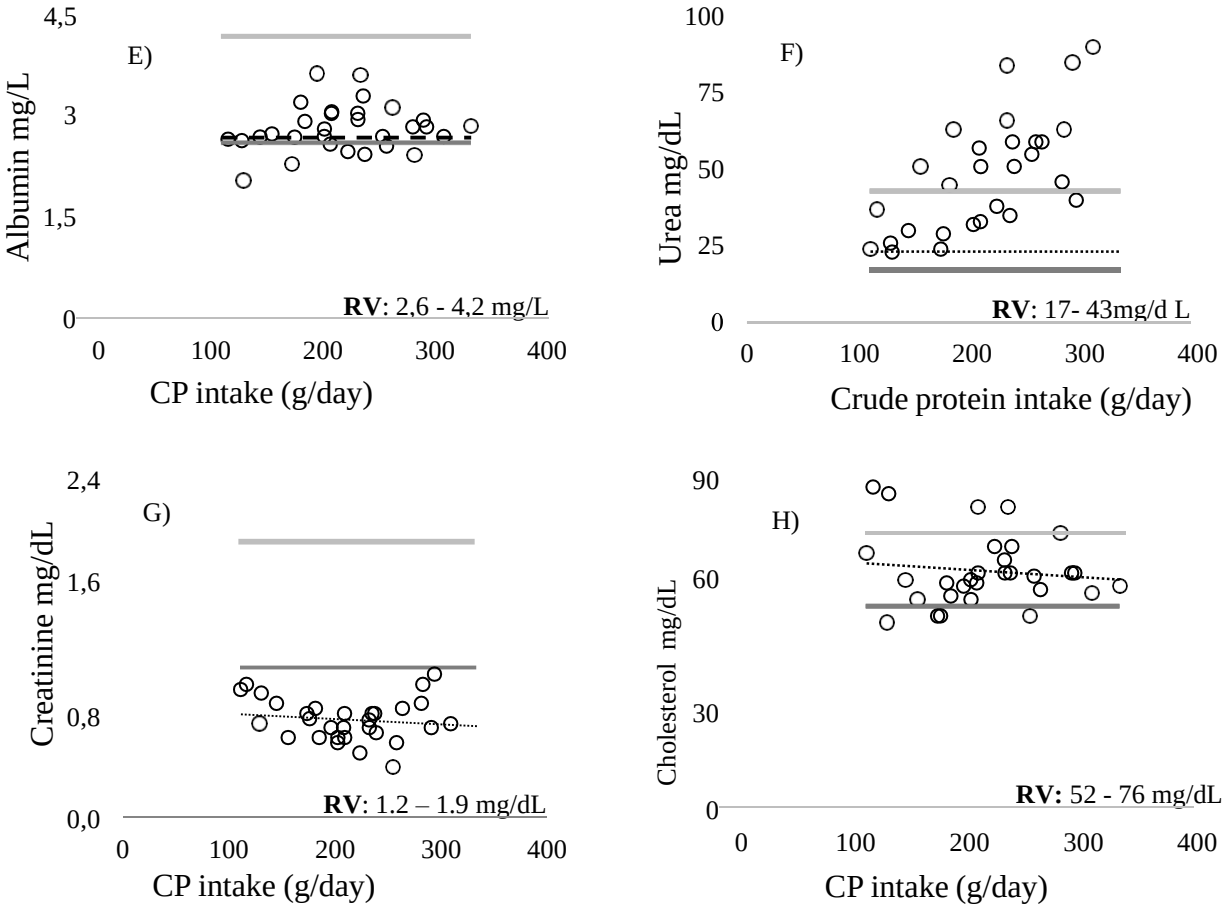

Figure 8. Relationship between crude protein (CP) intake (g/day) and the values of the metabolites E) Albumin, F) Urea, G) Creatinine, and H) Cholesterol in the serum of sheep, the light gray line represents the maximum value and the dark gray line the minimum value according to the reference values RV: maximum ( ), minimum( ) Gonzales and Silva (2022).

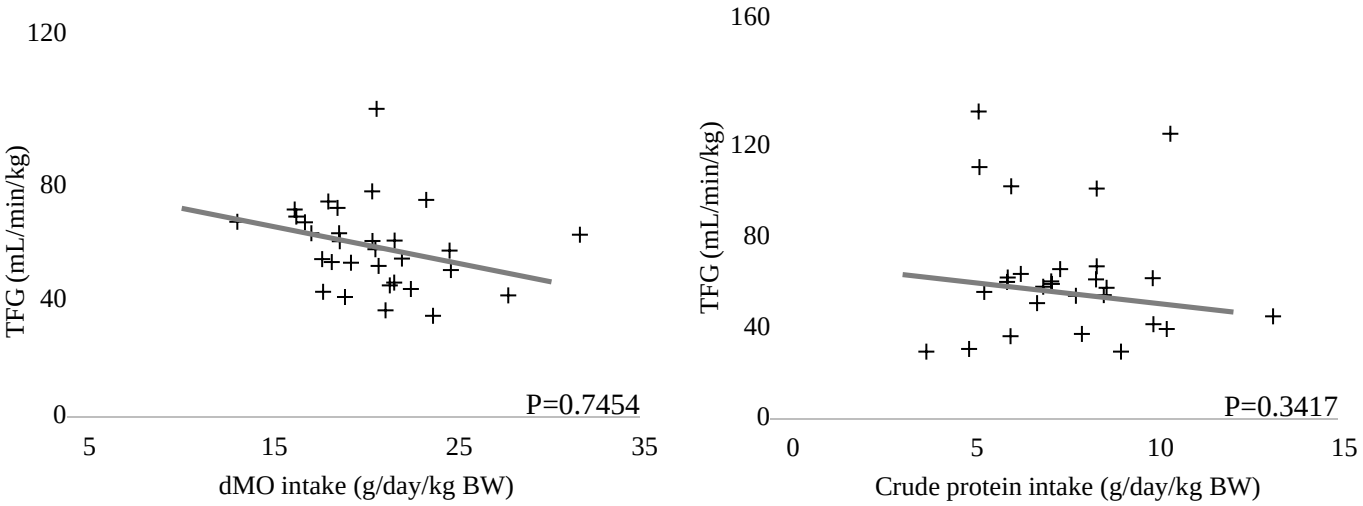

Figure 9. A) Relationship between digestible organic matter (dOM) intake (g/day/kg BW) and glomerular filtration rate (GFR) (ml/min/kg). B) Relationship between crude protein (CP) intake (g/day/kg BW) and glomerular filtration rate (GFR) (ml/min/kg).

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