Revista Electrónica:
Depósito Legal: ppi 201502ZU4665 / / ISSN electrónico: 2477-944X
Revista Impresa:
Depósito Legal: pp 199102ZU46 / ISSN 0798-2259

UNIVERSIDAD DEL ZULIA

REVISTA CIENTÍFICA

FACULTAD DE CIENCIAS VETERINARIAS
DIVISIÓN DE INVESTIGACIÓN

MARACAIBO, ESTADO ZULIA, VENEZUELA

Vol. XXX (3) 2020

REVISTA CIENTÍFICA

Fundada en 1990

Universidad del Zulia
Facultad de Ciencias Veterinarias
Maracaibo, Estado Zulia, Venezuela

Vol.XXX (3) 2020

La edición de esta revista ha sido auspiciada por el Vicerrectorado Académico LUZ, el Sistema de Servicios Bibliotecarios y de Información (Serbiluz) y Consejo de Desarrollo Científico y Humanístico de LUZ (CONDES)

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Revista Científica
Universidad del Zulia,
Facultad de Ciencias Veterinarias
Núcleo Agropecuario Ciudad Universitaria
Apdo.15252, Maracaibo 4005-A
Estado Zulia-Venezuela
Telf.-Fax:58-261-4126158
E-mail:revistafcv@gmail.com
http://www.fcv.luz.edu.ve

http://produccioncientificaluz.org/index.php/cientifica

El índice acumulado anual
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Esta revista fue editada en formato digital y publicada en Diciembre de 2020, por
La Facultad de Ciencias Veterinarias de La Universidad del Zulia
Maracaibo - Venezuela.

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Portada:

DESCRIPTION OF HUTMANNIN-1, A NEW PIII-METALLOPROTEASE FROM THE VENOM OF THE NEOTROPICAL LANSBERG'S HOGNOSE VIPER *(Porthidium lansbergii hutmanni)* WITH FIBRINO(GENO)LYTIC AND HAEMORRHAGIC ACTIVITIES

(Págs. 142 - 156)

Revista impresa
ISSN 0798-2259
Depósito Legal: pp 199102ZU46
Revista electrónica
Depósito Legal: ppi 201502ZU4665
ISSN electrónico: 2477-944X

www.luz.edu.ve

UNIVERSIDAD DEL ZULIA

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REVISTA INTERNACIONAL ARBITRADA DEDICADA A LA DIVULGACIÓN DE INVESTIGACIONES ORIGINALES EN EL ÁREA AGROPECUARIA

Vol. XXX (3) 2020
MARACAIBO, ESTADO ZULIA, VENEZUELA

Indizada y registrada en:
Institute for Scientific Information (ISI): Research Alert® y Focus on:
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La Revista edita 4 números anual.

Toda correspondencia debe ser enviada a:
Editor Jefe REVISTA CIENTÍFICA
Facultad de Ciencias Veterinarias
Universidad del Zulia. Apartado 15252.
Maracaibo 4005-A, Estado Zulia, Venezuela
Teléfono-Fax: (58-0261) 4126158.
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REVISTA CIENTÍFICA, is an international journal representing the scientific organ of the Faculty of Veterinary Sciences at the University of Zulia, Maracaibo, Zulia State, Venezuela. The aim is publishing the best tropical and subtropical scientific literature related to the fields of veterinary clinical sciences, animal production, public health, food sciences and technology of animal products as well as scientific literature generated in temperate zones but with tropical applicability. All the submitted manuscripts must follow the established editorial guidelines and go through an anonymous peer review process.

REVISTA CIENTÍFICA is published every two months.

All mail must be sent to:
Editor in Chief REVISTA CIENTÍFICA
Facultad de Ciencias Veterinarias
Universidad del Zulia. Apartado 15252.
Maracaibo 4005-A, Estado Zulia, Venezuela
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Artículo 2: La Orden al Mérito Universitario Dr. Francisco Ochoa se propone identificar, ponderar, valorar y premiar :

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REVISTA CIENTÍFICA

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Vol.XXX (3) 2020

Medicina Veterinaria / Veterinary Medicine. Pág.

EDITORIAL

Estar frente a la gerencia de una institución universitaria le permite vivir experiencias muy enriquecedoras, pero jamás pensé vivir en el presente año 2020 una situación tan difícil como la actual, no solo en lo nacional por la más profunda crisis, económica, política y social por la que atraviesa nuestra querida Venezuela, sino por la crisis de salud mundial producto de la Pandemia por el virus SARS-CoV-2, el cual produce la enfermedad COVID-19 (enfermedad por Coronavirus 19), situación que ha producido cambios impensables que van desde la forma de vida, hasta la propia prosecución académica a todos los nivel del proceso de enseñanza aprendizaje. Así mismo, la afectación de los procesos vitales en todas las instituciones de diferente índole.

Según la UNESCO, 1.500 millones de estudiantes en 165 países no pueden asistir a sus centros de enseñanza debido a la Pandemia por la enfermedad COVID 19. Dicha situación ha obligado a la comunidad académica internacional a explorar nuevas formas del proceso de enseñanza aprendizaje no presenciales, incluida la educación a distancia y en línea. Esta situación indudablemente también afecta el normal desarrollo de la actividad investigativa en institutos de investigación y universidades a nivel mundial, ya que se han tenido que cerrar temporalmente los laboratorios y suspender las actividades de campo en la búsqueda de resultados para resolver problemas planteados en innumerables investigaciones, lo cual sin duda tendrá un impacto negativo en la producción científica y humanística en todo el mundo. Solo en Latinoamérica la UNESCO reporta 24 millones de estudiantes y 1,4 millones de docentes que han sido afectados por la pandemia, lo que amenaza según la UNESCO en profundizar la desigualdad educativa que tiene la región.

En nuestra Facultad de Ciencias Veterinarias (FCV) de la Universidad del Zulia, la situación no ha sido diferente a lo planteado anteriormente, pues siendo Medicina Veterinaria una carrera práctica en aproximadamente un 80% de su contenido programático para la consecución de las competencias de acuerdo al perfil del egresado, ha tenido que ofrecer cursos virtuales de las asignaturas eminentemente teóricas (semestre especial) y suspender otros procesos y servicios que presta la FCV, entre ellos los laboratorios de investigación y servicio, reduciendo el funcionamiento a su mínima expresión, al punto de que solo está funcionando al mínimo la Policlínica Veterinaria Universitaria y la Farmacia Veterinaria, pues debemos ajustarnos al horario de restricción impuesto por el gobierno regional y nacional producto de las medidas de bioseguridad ante la pandemia que estamos viviendo.

Lo anteriormente expuesto es una realidad que ha tocado a nuestra celosamente protegida Revista Científica de la Facultad de Ciencias Veterinarias de la Universidad del Zulia, al punto que aunados esos dos grandes problemas (Pandemia y Situación País), nuestra revista se vio en la imperiosa e impostergable necesidad de bajar su frecuencia de bimestral, es decir seis (6) números por año, a una frecuencia trimestral (cuatro números por año). A pesar de esa decisión y producto de la pandemia por las implicaciones en las investigaciones a nivel mundial antes explicadas, se ha visto disminuido considerablemente el número de artículos recibidos para arbitraje durante los últimos 6 meses. A lo anterior se le suma un problema que está afectando a todas las revistas científicas en Venezuela y el mundo, de la cual la nuestra no es la excepción, como son las "revistas depredadoras" o "predatory journals", situación desconocida por muchos investigadores. En la Universidad del Zulia se han tomado acciones para tratar de frenar esta práctica que yo llamaría "fraude científico", sin que hasta la fecha se tengan resultados satisfactorios. Es por ello que conmino a todas los comités editoriales de Venezuela a formar un grupo de defensa de la producción científica de nuestros autores, para que en un frente común con otros países se detenga la depredación de las revistas científicas.

Dr. José Manuel Rodríguez Márquez
Decano FCV-LUZ. 2005-Actualidad

EFECTO DE LA HARINA DE AJÍ *(Capsicumm annuum Var. bremisculum)* SOBRE LOS INDICES PRODUCTIVOS DE POLLOS

EFFECT OF CHILI FLOUR (*Capsicumm annuum Var. bremisculum*) ON CHICKEN PRODUCTION ÍNDICES REPERCUSIÓN DE AJÍ EN LA SALUD DE LOS POLLITOS

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RESUMEN

El objetivo del estudio fue evaluar el comportamiento productivo de pollitos Cobb 500 en etapa 1-21 días (d) de edad en las condiciones andinas del Ecuador. Se emplearon un total de 315 pollos, con un d de edad, distribuidos en tres grupos de 105 animales cada uno: T1, Control (dieta basal sin aditivo); T2, dieta basal más 10 partes por millón (ppm) de ají (*Capsicumm annuum* Car. *bremisculum*) y T3, dieta basal más 50 ppm de ají. Se utilizó un diseño completamente aleatorizado donde se evaluó: grado de aceptabilidad, ganancia de peso (GP), ganancia media diaria (GMD), conversión alimenticia (CA), ocurrencia de diarreas, mortalidad y peso relativo de órganos linfoides. El grado de aceptabilidad fue superior (*P*<0,05) en los animales del tratamiento T3; la GP, GMD y CA (P>0,05) no variaron entre tratamientos; la ocurrencia de diarrea fue menor (*P*<0,05) en el grupo T3, en este mismo tratamiento (T3) no hubo mortalidad; el tamaño del timo y la bolsa de Fabricio fue superior (P<0,05) en los tratados con ají, de éstos el T3 tuvo mejor comportamiento. Se concluye que el uso de la harina de ají en pollos de 1-21 d de edad no mejora la GP ni la CA. El peso relativo de timo, bazo y bolsa de Fabricio no variaron entre tratamientos, mientras que el tamaño del timo y bolsa de Fabricio fue mejor en el tratamiento T3. Asimismo, se logró disminuir los trastornos diarreicos y la mortalidad.

Palabras clave: Aditivos alimentarios; ganancia de peso; mortalidad; pesos de órganos

ABSTRACT

The objective of the study was to evaluate the productive behavior of Cobb 500 chicks in stage 1-21 days (d) of age in the Andean conditions of Ecuador. A total of 315 one-d-old chickens were used, distributed in three groups of 105 animals each: T1, Control (basal diet without additive); T2, basal diet plus 10 parts per millions (ppm) of chili pepper (Capsicumm annuum var. Bremisculum) and T3, basal diet plus 50 ppm of chili. A completely randomized design was used where the degree of acceptability, weight gain (WG), mean daily gain (MDG), feed conversion (FC), occurrence of diarrhea, mortality and relative weight of lymphoid organs were evaluated. The degree of acceptability was higher (P<0.05) in the T3 treatment animals; WG, MDG and FC (P>0.05) did not vary between treatments; the occurrence of diarrhea was lower (P<0.05) in group T3, in this same treatment (T3) there was no mortality; the size of the thymus and Fabricio's bag was larger (P<0.05) in those treated with chili, of these T3 had better performance. It was concluded that the use of Chili flour in chickens 1-21 d of age does not improve WG or FC. The relative weight of the thymus, spleen and Fabricio's bursa did not vary between treatments, while the size of the thymus and Fabricio's bursa was better in treatment T3. Likewise, it is possible to reduce diarrheal disorders and mortality.

Key words: Food additives; weight gain; mortality; organ weights

Recibido: 01/01/2020 Aceptado: 06/07/2020

INTRODUCCIÓN

En la actualidad la inclusión de suplementos no sintéticos en la alimentación animal y humana ha sido prometedor; numerosos estudios [1-3, 15] demuestran efectos positivos de los aditivos como: probióticos, prebióticos, ácidos orgánicos y extracto de plantas [(comino (*Cuminum cyminum*), ajo (*Allium sativum*), nuez moscada (*Myristica fragrans*), jengibre (*Zingiber officinale*), canela (*Cinnamomum verum*) y ají (*Capsicumm annuum*)] sobre la salud del hospedador. En la década pasada, el uso de aditivos sintéticos como son los antibióticos promotores de crecimiento (APC) permitió lograr eficiencia económica y productiva en las industrias pecuarias [14]. Sin embargo, el uso indiscriminado de estos productos afecta negativamente en la salud del animal y del hombre [5, 15].

Los restos no degradados de los APC's se acumulan en los tejidos y en los productos finales (carne, leche, huevo), y al consumir estos productos se afecta la salud [5, 9]. Según, Guo y col. [9] aproximadamente el 60% de la producción avícola en el Ecuador emplean APC's en la producción. Pero, los restos no degrados pudiera estar presentes en los productos finales, y al consumirlos, se afecta la salud del consumidor [3,5].

Una de las alternativas para sustituir los APC's en las producciones pecuarias en la actualidad es la inclusión de extractos de plantas [2,21], saborizantes naturales [22], aceites esenciales [23], semillas [12, 14], microorganismos eficientes [8, 9, 15] entre otros aditivos en las dietas de los animales. En base a lo anterior, el uso de la harina de añadir Ají (*Capsicumm annuum* Var. *bremisculum)* en las aves (*Gallus gallus*) sería una de las mejores alternativas económicas, sobre todo por el aporte de micronutrientes y el control de las enfermedades [2, 8, 22, 23].

Algunos estudios [12, 17, 19] reportan efectos positivos de los *C. annuum* en la: a) prevención de la peroxidación [3], b) normalización la microbiota natural [1, 10], c) regeneración de los linfocitos T y B, macrófagos y células natural killer (NK) [11], d) sintetizan los interferones y e) normalizan el sistema inmune de los animales [19, 21, 22]. La inclusión de los capsaicinoides en la dieta de las aves reduce la carga de las enterobacterias en el tracto digestivo [17], en consecuencia, se consigue reducir los trastornos diarreicos, mejora la salud y los parámetros productivos [3, 11, 12].

La disponibilidad y costo de los extractos de plantas en países subdesarrollos es una limitante. Sin embargo, el uso de harina de ají (HA) obtenido de forma artesanal e incluida en la dieta en dosis mínimas pudiera resultar apropiado. Extractos de la planta de ají han sido evaluados y utilizados en pollos de engorde con resultados confortadores [17]. Un ejemplo de esto es la disminución de trastornos diarreicos y muertes, mejora la ganancia de peso (GP) y normaliza el sistema inmune [23]. En base a los antecedentes mencionados, el presente estudio tuvo como objetivo evaluar el comportamiento bioproductivo de los pollitos Cobb 500 en la primera etapa (1-21 días (d) de edad) productiva en las condiciones de zona andina del Ecuador.

MATERIALES Y MÉTODOS

Área de estudio

El trabajo experimental se realizó en la granja Irquis perteneciente a la Facultad de Ciencias Agropecuarias, Universidad Cuenca, Ecuador. La zona se encuentra en la parroquia Victoria del Portete, en el kilómetro (Km) 20 de la Vía Salado-Lentag. El lugar se encuentra a 2.671 metros sobre el nivel del mar (m.s.n.m.), presenta una precipitación promedio anual de 1078,05 milímetros (mm), temperatura mínima de 7°C y de máxima de 12°C.

Manejo experimental

Acondicionamiento del galpón: Previo a la recepción de los animales, se extremaron medidas de bioseguridad normados según Manual de Normas Básicas de Bioseguridad de una granja avícola [6], lo que permitió el control zoosanitario de los pollos durante el estudio; para el efecto se empleó glutarhaldeido, amonio cuaternario y alcohol isopropílico en dosis de 3 centimetros cubicos (cc^3) / litros (L) como desinfectante, según lo descrito por Radostits y col. [20].

Animales empleados: Se emplearon un total de 315 pollitos broiler Cobb 500, peso vivo (PV) 45 ± 2 gramos (g) pesados en una báscula digital (Camry, Ek2150, China) de 5 kilogramo (Kg) con error ± 0.25 g de capacidad, un d edad, procedentes de la incubadora "Incupasaje", El Oro, Ecuador.

Diseño experimental y tratamientos empleados: Los pollos fueron distribuidos al azar en nueve grupos experimentales de 35 animales cada uno. La HA se suministró a los pollos de los grupos T2 y T3 diariamente a las 07:00, como se describe en la TABLA I.

TABLA I

LOS TRATAMIENTOS EMPLEADOS EN EL ESTUDIO

Tratamientos	Material empleado
T1	Dieta basal sin harina de ají
T2	Dieta basal más 10 ppm de harina de ají por d
T3	Dieta basal más 50 ppm de harina de ají por d

Alojamiento y dieta basal: los pollitos estuvieron alojados en corrales colectivos de 2,5 metros cuadrado (m^2), piso de cemento y cama de cascara de arroz (*Oryza sativa*), con una densidad de 15 pollos por m^2. El alimento ofrecido estuvo compuesto por los siguientes ingredientes y se mezcló en la relación: 55,75; 35,20; 2,90; 2,10; 1,05 y 3%, maíz (*Zea mays*) molido; pasta de soya (*Glycine max*); aceite de palma (*Elaeis guineensis Jacq.);* fosfato dicalcico; carbonato de calcio y núcleo inicial campero,

respectivamente. La composición bromatológica de la dieta ofrecido a los pollos se describe en la TABLA II; los mismos que cumplen con los requerimientos nutricionales para las aves recomendados por el National Research Council (NRC) [18]. El agua fue ofrecida *ad libitum* en bebederos automáticos (Plasson, SKU: 885B722-8, Argentina) .

TABLA II

COMPOSICIÓN BROMATOLÓGICA DEL ALIMENTO OFRECIDO A LOS POLLOS

Componentes (%BS)	Alimentos	
	Dieta basal	Harina de ají
Materia seca	93	92
Proteína cruda	28,35	12,22
Proteína verdadera	22,25	8,23
Energía (MJ/Kg)	12.65	9.57
Extracto etéreo	4,52	5,31
Cenizas	3,35	3,21
Premezclas	0,86	-

BS: base seca

Manejo ambiental: la temperatura del ambiente y de la cama se mantuvo a 28 y 33 °C, respectivamente, durante los siete primeros d, luego se redujo en 3,0 °C cada semana (sem) hasta el final (21 d de edad). El fotoperiodo fue controlado según la edad, de uno a cinco d se proporcionó 23 horas (h) de luz y una de oscuridad, a partir de ahí, se redujo las h de claridad en una h cada 5 d hasta 21 d de edad. La humedad relativa de la nave se mantuvo en 68%. Las camadas de cada tratamiento se ubicaron distantes unas de otras, con un m de distancia en el intermedio a ambos lados del pasillo, para evitar la interferencia. Las aves en estudio recibieron las atenciones veterinarias oportunas según la Guía de Manejo del Pollo de Engorde [25].

Empleo de vacunas: A todos los animales en estudio se vacunaron contra el virus de Newcastle [cepa la Sota, Zoetix, Estados Unidos de América, (EUA)] y Gumboro (cepa intermedia, Zoetix, EUA), a los 8 d de edad, en forma individual con una gota al ojo y revacunando con la misma cantidad a los 12 d posteriores a la primera dosis.

Sacrificio de animales: Se seleccionaron cinco pollos por tratamiento, PV 600 ± 40 g, el sacrificio se realizó posterior a las 12 h de suspender la dieta basal, para dicho procedimiento se empleó la metodología descrita por Majó y Dolz [13], que cumple los principios de la bioética.

Preparación y obtención de la harina de ají

Material empleado y el procesamiento: la obtención de la HA se realizó en el laboratorio de Bioquímica, Carrera de Medicina Veterinaria, Facultad de Ciencias Agropecuarias, Universidad de Cuenca; para este estudio se utilizaron 5 kg en base húmeda de ají proveniente del Valle de Yunguilla, Santa Isabel, Azuay. Al material empleado se le quitaron las impurezas (polvo, restos de hojas, entre otros) mediante el lavado con agua al chorro, luego se procedió al secado a la temperatura ambiente (16 ± 1°C), a continuación se redujo el tamaño mediante el corte con un cuchillo (Tramontina, Brasil) hasta lograr una dimensión de 5 ± 1 centímetros (cm) seguidamente, se procedió a la etapa de deshidratación, para tal proceso se empleó una estufa (Memmert UN 30 PLUS, 1942794, Alemania) a 37 °C por 36 h, finalmente se molió en un molino eléctrico (Grinding Mill, Modelo 4e, México) de 20 kg de capacidad, hasta lograr obtener HA. Para conservar el producto obtenido se utilizaron frascos de cristal de boca ancha estériles, con capacidad de 1,0 kg, a temperatura ambiente de 16 ± 2 ºC, según la metodología descrita por Miranda y col. [16].

Variables evaluadas

Grado de aceptación: para evaluar la aceptación o el rechazo de la HA, se suministró HA, la cual fue ofrecida dos horas posterior al consumo de la dieta basal correspondiente a las 07:00 a.m. Para medir esta variable se empleó la siguiente escala: 1 = rechazo total; 2 = poco consumo; 3 = aceptación parcial; 4= aceptación total.

Indicadores productivos: Las aves fueron pesadas al inicio, a los 7; 14 y 21 d de edad; con esta información se calcularon la GP, la ganancia media diaria (GMD) y conversión alimenticia (CA), para este último, se empleó la siguientes formula: cantidad de alimento consumido /GP. Para el pesaje de los animales se utilizó una báscula electrónica (Camry, China) de 30 kg de capacidad con error de ±100 g.

Casos diarreicos y mortalidad: Se realizó un riguroso control clínico, según lo descrito en el manual de Normas Básicas de Bioseguridad de una Granja Avícola [4], para detectar cambios de conducta, trastornos diarreicos y muertes, lo cual facilitó determinar la ocurrencia de diarrea y el porcentaje de mortalidad.

Peso relativo de los órganos linfoides: Tras el sacrificio de los pollos, se practicó la disección para aislar algunos segmentos como el timo, el bazo y la bolsa de Fabricio, para dicho proceso se separó cuidadosamente del mesenterio, se lavó con agua destilada estéril y se pesaron en una balanza analítica de precisión (Scientech, Modelo 5A210I.W, China) de 3,100 ± 0,1 g de capacidad, según la metodología utilizada por García y col. [7]. Con esta información se calculó el peso relativo. La medición de los órganos linfoides se realizó (mm) con el empleo de calibrador digital electrónico (Hardened, Digital Calipers, China), con esta información se evaluaron el tamaño relativo.

Análisis estadístico

Los datos obtenidos en el estudio se procesaron con el paquete estadístico SPSS v. 22 para Windows [24]. Se realizó análisis de varianza según diseño completamente aleatorizado y, en los casos necesarios, se aplicó la prueba de rango múltiple

de comparación de Duncan [4] para discriminar diferencias entre medias a *P*<0,05.

Aspectos éticos: La recolección y utilización del material empleado (pollos, HA) fue de manera oportuna y sin generar ningún efecto dañino al ambiente. Se respetaron todos los protocolos para la manipulación de animales y conservación de las muestras.

RESULTADOS Y DISCUSIÓN

En la TABLA III se reporta los valores de la aceptación y/o rechazo de la HA incluido en la dieta basal de los pollos. Durante la primera etapa del estudio (siete d), no hubo diferencia (*P* >0,05) en cuanto a la aceptación de este aditivo en la dieta de los animales en estudio (T2 y T3). En la evaluación realizada a los 14 y 21 d de estudio, la aceptación del alimento con 50 ppm de ají (T3) fue mayor (*P* <0,05) que en el T2.

TABLA III

GRADO DE ACEPTACION DE LA HARINA DE AJÍ EN LOS POLLITOS

Días	Tratamientos		EE	*P-valor*
	T2	T3		
7	3,21	3,22	0,26	0,554
14	3,85	4,52	0,12	0,042
21	3,82	4,83	0,03	0,031

[a, b, c] letras distintas en la misma fila difieren a *P* <0,05, mediante la comparación de proporciones medias**. T2,** dieta basal + *Capsicumm annuum Var. bremisculum* a 10 ppm **T3,** dieta basal + *Capsicumm annuum Var. bremisculum* a 50 partes por millon (ppm). **EE.** error estandar

En la TABLA IV se refleja las medias de GP y CA en las diferentes edades. La GP, evaluados a los 7; 14 y 21 d de edad no presentaron diferencias entre tratamientos (*P* <0,05). A los siete d de edad, la CA no varió (P=0.621) entre grupos. Sin embargo, en la evaluación realizada a los 14 y 21 d de estudio, los animales que consumieron diferentes dosis de ají (T2 y T3) tuvieron mayor CA que en el tratamiento control (*P* <0,05).

TABLA IV

INDICADORES DE LA GANANCIA DE PESO Y CONVERSION ALIMENTICIA DE LOS POLLOS, SUPLEMENTADOS CON LA HARINA DE AJÍ

Días	Indicadores	Tratamientos			EE	
		T1	T2	T3		
7	GP, kg	0,17	0,17	0,17	0,06	0,872
	CA, U	1.06	1.03	1.08	0,05	0,621
14	GP, kg	0,38	0,36	0,36	0,02	0,951
	CA, U	1.25[b]	1.32[a]	1.23[b]	0,05	0,041
21	GP, kg	0,63	0,59	0,57	0,03	0,532
	CA, U	1.29[b]	1.35[a]	1.37[a]	0,02	0,034

[a,b,c] letras distintas en la misma fila difieren *P* <0.05 (Duncan, 1955). **T1,** Dieta basal sin aditivo. **T2,** dieta basal + *Capsicumm annuum Var. bremisculum* a 10 ppm **T3,** dieta basal + *Capsicumm annuum Var. bremisculum* a 50 ppm. **GP,** ganancia de peso. **CA.** Conversión alimenticia. **EE,** Error estándar.

En la TABLA V se reporta el comportamiento de la salud (ocurrencia de diarreas y % mortalidad) en la primera etapa productiva (a partir de uno a 21 d de edad). En los animales de los grupos (T2 y T3); que consumieron dietas que contenían diferentes dosis de ají presentaron menor ocurrencia de trastornos diarreicos y el porciento de mortalidad fue inferior a 1.5 %, con respecto a los animales del grupo control.

TABLA V

OCURRENCIA DE DIARREAS, MORTALIDAD EN POLLOS EN LA ETAPA DE ENGORDE AL INCLUIR HARINA DE AJÍ EN LA DIETA BASAL

Días	Indicadores, %	Tratamientos			EE	*P-valor*
		T1	T2	T3		
7	Ocurrencia de diarreicos	5,12[a]	2,54[b]	1,10[c]	0,02	0,012
	Mortalidad	4,04	1,01	-	-	-
14	Ocurrencia de diarreicos	5,86[a]	2,23[b]	1,03[c]	0,05	0,001
	Mortalidad	2,08	1,02	-		
21	Ocurrencia de diarreicos	5,05[a]	2,35[b]	0,04[c]	0,01	0,001
	Mortalidad	-	1,03	-	-	-

[a,b,c] letras distintas en la misma fila difieren *P* <0.05 (Duncan, 1955). **T1,** Dieta basal sin aditivo. **T2,** dieta basal + *Capsicumm annuum Var. bremisculum* a 10 ppm **T3,** dieta basal + *Capsicumm annuum Var. bremisculum* a 50 ppm. **EE,** error estándar.

En la TABLA VI se presenta el peso relativo y tamaño de los órganos linfoides de los pollos tratados con ají. El peso del timo, el bazo y la bolsa de Fabricio de los pollos con 21 d de edad, no difirieron (*P* >0,05) entre tratamientos. Mientras que, en el grupo T3, el tamaño del timo y la bolsa de Fabricio fue superior (P<0.05) que en los demás tratamientos.

TABLA VI

COMPORTAMIENTO DE PESO Y TAMAÑO DE LOS ÓRGANOS LINFOIDES, AL SUPLEMENTAR AJÍ, (*Capsicumm annuum* var. *bremisculum*)

Órganos linfoides	Indicadores	Tratamientos			EE	*P-valor*
		T1	T2	T3		
Timo	Peso, g	1,7	1,61	1,65	0,02	0,542
	Tamaño, mm	66,3[b]	70,2[a]	70,6[a]	0,05	0,042
Bazo	Peso, g	0,51	0,56	0,56	0,03	0,532
	Tamaño, mm	12,4	12.1	12,7	0,02	0,862
Bolsa de Fabricio	Peso, g	1,2	1,3	1,6	0,03	0,578
	Tamaño, mm	16,2[b]	15,4[c]	17,6[a]	0,02	0,032

[a,b,c] letras distintas en la misma fila difieren *P* <0.05 (Duncan, 1955). **T1,** Dieta basal sin aditivo. **T2,** dieta basal + *Capsicumm annuum Var. bremisculum* a 10 ppm **T3,** dieta basal + *Capsicumm annuum Var. bremisculum* a 50 ppm. **EE,** errar estándar.

Aceptación y rechazo al consumo de la HA en pollos jóvenes: los animales que aceptaron y consumieron la HA inoculado en la dieta basal, se comenzó a reflejar una pequeña mejoría a partir de siete d edad, los resultados obtenidos en el estudio podrían estar relacionado posiblemente con la edad y desarrollo fisiológico del animal, a medida que crece los pollitos requiere mayor cantidad de nutrimientos para suplir el mantenimiento y la producción (5, 7, 17].

Comportamiento productivo: la no variación en cuanto al indicador GP en los pollos jóvenes (entre 1-21 d de edad) en el presente estudio, posiblemente se deba a que la inclusión mínima (10 y 50 ppm) de la HA en la dieta de estos animales. Los valores obtenidos en el estudio, coincide en parte con lo reportado por Zhai y col. [23], lo cual no favoreció a la mejora del GP en esta etapa productiva. En este sentido, Londoño y col. [11] demuestran que la inclusión de hasta 0,3% de ají en la dieta basal podría mejorar la GP hasta 300 g en pollos de engorde. Similares resultados reportan otros estudios Orndorff y col. [19]; Lozada [12]; Morales y Murillo [17] y Dahloum y col. [1], con el empleo de hasta 500 ppm, quienes logran mejorar el comportamiento productivo. Así mismo, Lozada [12] con el uso de 500 ppm HA durante 14 d de edad reportó mejoras en el comportamiento productivo. Sin embargo, la dosis empleada en este experimento fue 50 ppm, lo que representa ínfima con respecto a otros reportes, lo que justificaría en parte la no mejora de GP en pollos jóvenes.

En el presente estudio, los pollos que consumieron HA en dosis mínimas a los 14 y 21 d de edad presentaron una mejor CA con respecto a los animales del grupo control, lo cual posiblemente intervino en la activación de los principales aminoácidos estructurales, sobre todo con las de la conformación de las vellosidades intestinales, debido a que en esta etapa el animal está en pleno desarrollo fisiológico. Además, los resultados de esta variable son dependientes del efecto integridad intestinal, el empleo de aditivos en animales en ayunas (ingesta de primeras h de la mañana) brinda una mayor absorción. Los valores obtenidos en el presente estudio se asemejan con los reportados por Meredi y col. [14]; Guetiye y col. [8]; Díaz y col. [2], quienes con el uso de 500 ppm de ají obtuvieron una mejor CA en pollos de engorde. Todo lo anterior, contribuye a la mayor asimilación de los principales nutrientes y menor depreciación de la dieta ofrecida, como resultado a este proceso ocurre mayor CA. Los resultados obtenidos concuerdan con los reportados por Sanabria y col. [21], quienes reportaron 1,19 de CA al emplear albahaca (*Ocimum basilicum*) (600 ppm), uno de los aditivos utilizados para la motilidad de las células entéricas. El uso de suplementos de origen vegetal como es la HA en la industria avícola, también podría mejorar algunos indicadores del comportamiento productivo en las diferentes especies de animales de interés zootécnico [11].

Comportamiento de salud: la menor ocurrencia de diarreas en los animales de los grupos tratados (T2 y T3), posiblemente fue debido a que estos aditivos naturales actúan sobre el movimiento de iones Na^{+}y K, quienes siguen la gradiente osmótica en la pared intestinal [2, 7, 23]. Miranda y col. [15] mencionan que el uso de aditivos naturales como: las enzimas, aceites esenciales y microorganismos probióticos son capaces de normalizar el sistema inmune, en consecuencia, mejora la salud del tracto digestivo y prepara para enfrentar de forma positiva a posibles agresiones de los agentes patógenos [1, 8, 16, 22]. Similares resultados a los obtenidos en el presente estudio también fueron reportados por Lozada [12]; Morales y Murillo [17]; Londoño y col. [11], pero en estos estudios los autores emplearon 500 ppm de ají. A pesar de emplear dosis muy inferiores con respecto a otros estudios, los resultados del presente trabajo se asemejan con los alcanzados por Orndorff y col. [19] y Lozada [12]. Por su parte, Korošec y col. [10]; Organización de las Naciones Unidas para la alimentación y la Agricultura (FAO) [5]; Miranda y col. [15]; Djellout y col. [3] y Dahloum y col. [1] mencionan que la inclusión de aditivos microbianos, extractos de plantas y algunos aceites esenciales son capaces de variar la producción de ácidos orgánicos sobre, todo a los de la cadena corta (ácido láctico y acético) a nivel intestinal, estos cambios favorecen a la reducción de los valores de pH, los cambios repentinos de los niveles de jugos gástricos a nivel intestinal limitan e eliminan los agentes patógenos generadores de diarreas (*Escherichia coli*, *Salmonella* spp., entre otros) en animales jóvenes.

CONCLUSIONES

La inclusión de 10 y 50 ppm de la HA en la dieta de pollos con 1-21 d de edad no mejora la GP ni la CA. El peso relativo de timo, bazo y bolsa de Fabricio no variaron entre tratamientos,

mientras que, el tamaño del timo y bolsa de Fabricio fue mejor en el tratamiento T3. Asimismo, se logra disminuir los trastornos diarreicos y la mortalidad.

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PRODUCTIVE PERFORMANCE OF THE "GREEN TERROR" *(Andinoacara rivulatus)* FISH DURING THE FATTENING STAGE WHEN FED DIETS WITH PASSION FRUIT CAKE *(Passiflora edulis)*

RENDIMIENTO PRODUCTIVO DE LA ESPECIE DEL PEZ NATIVO "TERROR VERDE" (*Andinoacara rivulatus*)" DURANTE LA ETAPA DE ENGORDE CUANDO SE ALIMENTA CON DIETAS CON TORTA DE MARACUYÁ *(Passiflora edulis)*

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ABSTRACT

The objective of the present work was to evaluate the effect of diets containing passion fruit cake on the productive performance of the "green terror" (*Andinoacara rivulatus*)". Fish were fed four passion fruit diets (T1: 0%, T2: 3%, T3: 6%, T4: 9%) for 30 days. The weight increase (WI), relative weight increase (RWI), growth rate (GR), incremental growth rate (IGR), feed conversion ratio (FCR), dry matter digestibility, gross protein digestibility (GPD), gross energy digestibility (GED), protein efficiency rate (PER), productive value of protein (PVP), and feed cost were assessed. There were no differences in the final weight, WI, RWI, GR and IGR values between T1 and T2; only the diet in T3 and T4 showed poorer results than the T1 diet. FCR was higher in the control diet (T1) and in T4 than in T2 and T3. As the percentage of passion fruit increased, the cost of the diet decreased. GPD and GED did not exhibit differences in T1, T2 and T3, only DT T4 had lower digestibility values. PER of control DT T1 had a better coefficient than T2, T3 and T4. Significant differences were found in PVP between the control DT, T2 and T4. However, the lowest values were obtained with this latter DT. The addition of up to 3-6% passion fruit did not affect the yield, and the cost of the diet was significantly reduced.

Key words: *Andinoacara rivulatus*; apparent digestibility; fattening stage; experimental diets; *Passiflora edulis*

RESUMEN

El objetivo fue evaluar el efecto de las dietas, que contenían maracuyá, en el rendimiento productivo del "terror verde". Los peces fueron alimentados con cuatro dietas de torta de maracuyá (T1: 0%, T2: 3%, T3: 6%, T4: 9%) durante 30 días. El aumento de peso (WI), aumento de peso relativo (RWI), tasa de crecimiento (GR), tasa de crecimiento incremental (IGR), conversión de alimento (FCR), digestibilidad de materia seca, de la proteína bruta (GPD) y de la energía bruta (GED), tasa de eficiencia proteica (PER), valor productivo de la proteína (PPV) y el costo de alimentación fueron evaluados. No hubo diferencias en los valores de peso final, FW, WI, RWI, GR e IGR entre T1 y T2; solo la dieta en T3 y T4 mostró resultados peores que la dieta T1. El FCR fue mayor en la dieta control (T1) y en T4 que en T2 y T3. A medida que el porcentaje de racuyá aumentaba, el costo de la dieta disminuía. GP y GE no mostraron diferencias en T1, T2 y T3, sólo el ensayo T4 tuvo valores de digestibilidad más bajos. El PER del ensayo de control tenía un coeficiente mejor que T2, T3 y T4. Se encontraron diferencias significativas en el PVP entre el ensayo de control, T2 y T4. Sin embargo, los valores más bajos se obtuvieron con este último ensayo. La adición de hasta 3-6% de maracuyá no afectó el rendimiento, y el costo de la dieta se redujo significativamente.

Palabras clave: *Andinoacara rivulatus*; digestibilidad aparente; fase de engorde;dietas experimentales; *Passiflora edulis*

Recibido: 01/07/2020 Aceptado: 26/08/2020

INTRODUCTION

Currently in Ecuador, there is no constant and adequate supply of plant resources for high protein certified organic preparation of diets for fish farming, highlighting the need to explore alternative plant resources (PR) to replace conventional protein sources, such as passion fruit cake (PFC) [18, 22, 31].

Organic aquaculture differs from conventional aquaculture in that it is focused on production in harmony with the environment, employing practices that seek to duplicate the natural conditions of organisms [8], striving always be committed to social, economic and sustainability factors, including the rational use of resources for feed [31].

The high cost of traditional energy products used in animal feed has sparked the search for new products and an evaluation of their nutritional potential. One of these crops is PFC, which is not well known, though it has great potential in feeding animals due to its very low cost.

PF is a source of protein, minerals, carbohydrates and fats. PF has an energy value of 78 calories, 2.4 grams (g) of carbohydrates, 5 g of calcium, 17 miligram (mg) of phosphorus, 0.3 mg of iron, 684 mg of activated vitamin A, 0.1 mg of vitamin B2 (riboflavin), 2.24 mg of niacin and 20 mg of vitamin C. In Ecuador there are around 28 thousand hectares (hes) planted PF with an average yield of about 14 tons (T) per hes. The main variety is *Passiflora edulis flavicarpa* (yellow fruit), as its production per hes is higher and it is ideal for processing. It is estimated that a well-managed plantation can yield 8-10 T per hes in the first year (yr), 15-20 T in the second yr and 12-14 T in the third yr [2].

Duchi and Pazmiño [10] have showed that the industrialization of PF produces by-products such as PF peel. Originally, these industrial by-products were solid waste that contaminated the environment (soil, air and water); however, advances in alternative animal production methods in the tropics have permitted fresh and dry peel to be used as a supplement in dairy (Bos-taurus) and beef cattle diets *Bos indicus.*

Mazón [21] has reported that PFC has a high content of dry matter (DM), a general average of 92.85% and an average value of 67.32% organic matter (OM). The gross protein (GP) and ether extract (EE) showed high values of 23.95 and 11.81%, respectively. Gross fiber (GF) and nitrogen-free extract (NFE) had an average value of 46.27 and 20.98%. The average values of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were therefore high, at 72.47 and 69.29%. The average ash content was 2.07%, and the general average for calcium and phosphorus was 2.85 and 0.32%. The average value of gross energy (GE) was 5.19 mega calories per kilogram of dry material (Mcal·kg $^{-1}$ DM).

The development of high nutritional quality, low environmental impact and economically profitable diets for fish farmers is a pressing need in the fish feed industry, especially for intensive production systems. Rations having these characteristics are possible when formulated with ingredients of high nutritional value, based on the ingredient digestibility data of each particular species [17].

The rapid expansion of fish farming in recent yr, like other forms of intensive animal production, requires improved nutrition as well as complete rations [13]. Feed is the most significant production cost; the increased complexity of the feed required in aquaculture means that this item normally exceeds 70% of the total cost, and therefore justifies the efforts to understand the principles of fish nutrition and feeding [1, 9].

Green terror (*Andinoacara rivulatus*), the target species of this work, is native to Peru and Ecuador. Males can reach 30 centimeter (cm), while females do not exceed 20 cm. They prefer rather high temperatures, not below 25 °C. They are very adaptable to pH and water hardness conditions, but they do not tolerate the presence of nitrogenated compounds in the water (such as ammonium); it is thus essential to change the water continuously and have a good filtration system when they are grown in captivity. It is an omnivorous fish, so it accepts any type of food. However, due to their voracity, they should not be overfed, as they will always seem dissatisfied. They are territorial and aggressive, so they should be housed in large ponds and never share the same place with smaller fish [22].

Passion fruit cake (PFC) has been used for fish, poultry and ruminants feeding programs as a source of protein and energy in monogastric and ruminant diets. In the case of monogastrics, it is used to replace fishmeal as the source of animal protein and corn (*Zea mays*) as the energy source, as both products are more expensive. There are reports of research into the use of PFC in the diets of native fish such as *Andinoacara rivulatus* to replace industrial fishmeal as the source of protein, and yellow corn as the source of energy.

Therefore, with the aim of expanding the knowledge of PFC and its uses in animal feed, this study evaluated the effect of diets based on PFC on the yield of the native “green terror” in the fattening stage during the dry season.

MATERIAL AND METHODS

Fish housing, feeding and sampling

Four hundred fish with an initial weight of 44.2 ± 1.5 g were used, and the stocking density was 25 fish per cage (1.11kg/meter (m^3)). There were 16 experimental cages 1 m long x 1 m wide x 1 m high, covered with 6 x 8 cm extruded plastic mesh, and the tank was 30 x 20 m and 0.8 m deep. An electric balance of 5 kg capacity and a minimum weight of 1 g was used for the

biometric data of the fish. A 30 x 1.5 m platform was used to deliver the experimental diets. The pond did not have aeration equipment because it was supplied with a constant 15 liter (L)/ minutes (min) flow of highly oxygenated, double filtered water from a 0.25 hes reservoir. The water temperature was 20 ± 0.5 °C. The dissolved oxygen content of the water in the tanks was maintained at 11 ± 1 mg/L and the pH was 7.5 ± 0.5. Nitrogenated wastes (unconsumed food and excreted organic matter) were removed daily using a Monge drainage system.

The fish had seven days (d) o adapt to the experimental cages before the start of the experiment and were fed high-protein extruded feed to accustom them to consuming concentrated feed. Four experimental diets were prepared with different percentages of PFC (0, 3, 6 and 9%) formulated by the trial and error method to represent isoproteic (35% total protein) and isocaloric (3000, 3006, 3006 and 3013 kilocalories (Kcal)/kg) diets, using the inputs and quantities shown in TABLE I. The experimental diet was offered *ad libitum* four times a d (0800, 1100, 1300 and 1500 hours (h) for 30 d. As a prophylactic measure, the fish were treated with a solution of methylene blue (5g/10 L every 30 d) to prevent *Ichthyopthirius* fungus and pathogenic bacteria. After each sampling, a solution of methylene blue diluted in water was used to prevent contamination by fungi and bacteria.

Dietary Treatments (DT)

The experimental DT were: T1 (0%), T2 (3%), T3 (6%) and T4 (9%), depending on the percentage of PFC in the diet. Experimental diets underwent a pelleting process involving agglomeration through the addition of binders such as bentonite and molasses, and water. Then they were passed through a fine diameter matrix to form spherical particles (pellets), which were hardened by steam cooking in a rotary kiln. A completely randomized design with four DT were used (4 diets with different percentages of PFC, four repetitions (4 cages) per DT, and the data were collected for 30 d. The manual on Nile Tilapia (Oreochromis niloticus) nutritional needs by Torres-Novoa [30], University of The Llanos, Colombia, was used as a reference, based on the inputs and the amounts indicated in TABLE I.

The experimental DT (diets) and their proximal composition of experimental diets are shown in TABLE II.

Determination of biological parameters

The ingredients, diets and feces were analyzed to determine their proximal composition according to the methodologies of Official Methods of Analysis [25], dry matter (DM) by kiln

TABLE I

COMPOSITION OF EXPERIMENTAL DIETS CONTAINING PASSION FRUIT CAKE USED IN THE FATTENING PHASE OF *Andinoacara rivulatus*

	Experimental diets (%)			
Ingredients (%)[1]	T1	T2	T3	T4
Corn	12.90	5.80	3.40	2.70
Wheat bran	0.00	2.00	3.00	0.00
Rice flour	11.50	16.00	17.30	8.10
Soybean cake	32.00	37.60	38.00	43.50
Fishmeal	36.00	30.00	28.00	27.00
Soybean oil	3.80	1.80	0.50	6.00
Passion fruit cake	0.00	3.00	6.00	9.00
Salt	0.10	0.10	0.10	0.10
Antifungal [2] Methionine	0.05	0.05	0.05	0.05
Antioxidant [3]	0.20	0.20	0.20	0.20
Choline Chloride	0.05	0.05	0.05	0.05
Bentonite	0.10	0.10	0.10	0.10
Pre-mix [4]	3.00	3.00	3.00	3.00
Enzyme [5]	0.10	0.10	0.10	0.10
	0.10	0.10	0.10	0.10

[1]Air dried food; T1: 0% Passion Fruit Cake, T2: 3% Passion Fruit Cake, T3: 6% Passion Fruit Cake, T4: 9% Passion Fruit Cake
[2] Mollejosanitin
3 Endox
[4] Rovimix Pre-mix: Vitamin A, D3, K, E, B1, B2, B6, Nicotinic Acid, Calcium Pantothenate, Biotin, Folic Acid, Choline, Inositol and Vitamin C
[5] Avizyme 1502 (600 U g^{-1} endo-1,4 beta xylanase EC 3,2,1,8; 8000 U g^{-1} subtilisin - protease- EC 3,4,21,62; 800 U g^{-1} alpha amylase EC 3,2,1,1.).

TABLE II

PROXIMAL COMPOSITION OF EXPERIMENTAL DIETS WITH PASSION FRUIT CAKE USED FOR THE JUVENILE PHASE OF *Andinoacara rivulatus*

Proximal composition (%)	T1	T2	T3	T4	Request[1]
Digestible dietary energy (Kcal kg-[1])	3000	3002	3003	3014	3000
Total protein	35.00	35.00	35.00	35.00	35.00
Fiber	3.10	4.20	4.90	6.30	---
Calcium	1.84	1.58	1.49	1.47	1.00
Phosphorus [2]	1.50	1.40	1.40	1.30	0.80
Arginine	1.91	1.96	1.98	2.36	0.94
Lysine	2.09	2.03	1.98	2.36	0.94
Met + Cys	1.11	1.09	1.08	1.12	0.35
Tryptophan	0.36	0.36	0.36	0.39	0.30

[1] Total phosphorus in the diet

drying at 105°C/24 h, gross protein (% N x 6.25) by the Kjeldahl method, lipids by solvent extraction with soxlhet apparatus, ash by incineration at 550°C/6 h and gross energy using a Parr Instruments 121AE adiabatic calorimetric pump (USA).

The experiment was carried out in a fattening pond where the 16 experimental cages containing 25 fish each were located, to which the PFC diets were fed to establish the productive yield of the native fish. Once the fattening period was over, the fish were transferred to metabolic tanks and introduced into each of them for processing.

To determine the digestibility of the diets, the total stool collection method was used in each of the DT and repetitions based on the modified Guelph system [28]. A cylindrical tank with a conical bottom containing 200 L of water was used; oxygen was provided by means of a portable oxygenator located at the top of the tank, and at the bottom, a 20 cm long tube of two inches in diameter length with a ball valve in the middle and a screw cap on the end was inserted, which allowed the feces to be collected every 6 h over a total period of 24 h [16]. The feces obtained in each collection were placed in a 50: cubic centimeter container, the excess moisture was removed and they were stored in a refrigerator (Indurama, Ecuador) at 4 °C until the corresponding bromatological chemical analysis was performed.

The yield of *Andinoacara rivulatus* was evaluated by measuring following zootechnical parameters.

Feed conversion ratio (FCR):

$$FCR = \frac{\text{Amount of feed distributed (g)}}{\text{Weight gain of the fish (g)}}$$

Weight increase (WI) is the weight increase per unit of time due to food consumption (energy and protein) at a certain temperature.

$$WI = (Wf - Wi)$$

Where:
Wf = final weight
Wi = initial weight

The relative weight increase (RWI) is the increase in weight per unit of time at different ages due to food consumption (energy and protein) within a given temperature range.

$$RWI\ (\%) = 100\ x \frac{(Wf - Wi)}{(Wi)}$$

Where:
Wf = final weight
Wi = initial weight
The growth rate (GR) is a measure of the average increase or decrease in weight over a period of 30 d due to the amount of feed consumed and the water temperature.

$$\mathrm{GR}\,(\%) = 100\,x\frac{(\mathrm{Wf}-\mathrm{Wi})}{t}$$

Where:
Wf = final weight
Wi = initial weight
t = time

The incremental growth rate (IGR) is a measure of the average weight gain over a period of 30 d due to the amount of feed consumed and the water temperature.

$$\mathrm{IGR}\,(\%) = \ 100\,x\frac{(ln\,Wf - ln\,Wi)}{t}$$

Where:
Wf = final weight
Wi = initial weight
t = time

Net feed consumption (NFC) is the amount of complementary feed consumed weekly minus the amount of residual feed of the measure weighed weekly:
NFC = Weight of feed consumed (g) - Weight of waste (g)

The protein efficiency rate (PER) is the weight gained by an animal for each unit of protein in the feed and was calculated for each DT using the following equation [3]:

$$PER = \frac{Weight\ gained}{Protein\ intake\ (g)}$$

The protein production value (PVP) were calculated for each assay using the following equation:

$$PVP = \frac{Protein\ retained\ (g)}{Protein\ intake\ (g)}$$

Apparent digestibility coefficient

The apparent digestibility coefficient (DC) was calculated by collecting all the feces in each of the DT and repetitions using the modified Guelph system [28]. This method employs a cylindrical tank with a conical bottom (metabolic aquarium) where water and oxygen are fed continuously from the top and there is a feces collection tube at the bottom [1, 4, 14, 15]. Subsequently, a bromatological analysis was performed on the feces extracted and samples of the experimental diets used in the studies.

The digestibility of the experimental fish diets was determined by the direct method, also called the total collection method. It consisted of the quantitative collection and analysis of all feces produced. The digestibility coefficient (DC) was calculated as follows:

$$.\% = \frac{Ingested\ nutrients - nutrients\ in\ feces}{Ingested\ nutrients}\,x$$

Statistical analysis

A completely randomized design was used with four DT and four repetitions. All zootechnical and biological parameters were analyzed with an ANOVA analysis of repeated measurements using the General Linear Model (GLM) of the Statistical Analysis System Software statistical package (Workflow Studio 1.3) System for Windows 11, Copyright 2016 by SAS Institute Inc., Cary, NC, USA). The model considered the percentage of PFC (0, 3, 6 and 9%), analyzed as repeated measurements on the same experimental units. When significant differences were detected between the means of factors with more than two levels, they were subjected to a multiple comparison of means using Honestly-significant-difference (HSD) of Tukey values expressed as mean ± standard error of mean (SEM).

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The unrestricted, randomized experiment consisted of four DT, four repetitions and 25 fish per experimental cage:
The mathematical model is shown below:

Yij = μ + Ti + εij
Where:
Yij = Observations for dependent variables.
μ = Average population
Ti = “i-th” effect of the DT
εij = Random effect (Experimental error).
The Tukey test was used for the comparison of means, with a probability of 5%.

RESULTS AND DISCUSSION

Digestibility of experimental diets

By feeding experimental diets based on PFC, it was possible to establish the digestibility coefficients of DM, GP and GE for this tropical native species during the fattening period, TABLE III.

When comparing the control diet T1 with diets T2, T3 and T4, significant differences were found in the apparent digestibility coefficients of the dry material ($P \leq 0.05$), but no significant

TABLE III
APPARENT DIGESTIBILITY COEFFICIENTS (DMD, GPD AND GED) FOR *Andinoacara rivulatus* JUVENILES FED DIETS CONTAINING PASSION FRUIT CAKE

	Dietary Treatments (DT)			
Variable[1]	T1	T2	T3	T4
Dry matter digestibility (%).	58.65 ± 0.35 **a**	58.20 ± 0.35 **b**	57.35 ± 0.35 **c**	50.58 ± 0.35 **d**
Gross protein digestibility (%).	84.48 ± 0.75 **a**	84.53 ± 0.75 **a**	84.42 ± 0.75 **a**	79.58 ± 0.75 **b**
Gross energy digestibility (%).	77.58 ± 0.69 **a**	77.10 ± 0.69 **a**	77.45 ± 0.69 **a**	71.70 ± 0.69 **b**
Protein efficiency rate (%)	0.33 ± 0.02 **a**	0.31 ± 0.02 **b**	0.26 ± 0.02 **c**	0.20 ± 0.02 **d**
Productive value of protein (%).	0.60 ± 0.004 **a**	0.59 ± 0.004 **b**	0.59 ± 0.004 **ab**	0.56 ± 0.004 **c**

[abc] Averages with different letters in the same line differ statistically according to the Tukey test
[1] These are the means (± EEM) of 25 fish housed in a cage (Experimental Unit) with four replicates per trial. T1: 0% Passion Fruit Cake, T2: 3% Passion Fruit Cake, T3: 6% passion fruit cake and T4: 9% passion fruit cake.

differences ($P \geq 0.05$) were found between DT T1, T2 and T3 and DT T4 in the digestibility of GP and GE. Moreover, this latter DT, which had the highest percentage (9% PFC), had the lowest digestibility coefficient compared to the other DT ($P \leq 0.05$).

The results obtained in this study agree with Vásquez *et al*. [31] in a study of tilapia (*Oreochromis* spp) and Vasquez *et al*. [32] for cachama (*Piaractus brachypomus*). The apparent digestibility coefficients achieved in this study are generally consistent with those described in other studies involving tilapia (*Oreochromis niloticus*) and cachama with similar DM, GP and GE. There are discrepancies among the results published in the literature, and according to a consensus among researchers, this may be caused by differences in the methodologies used to determine the coefficients or process the diets, differences in the amounts added of the ingredient being studied, the type of control diet used Anderson *et al*. [6], Boscolo *et al*. [8], Furuya *et al*. [13], Guimarães *et al*. [15], Masagounder *et al*. [20] the method of fecal collection Meurer *et al*. [23], the size of the fish, the equation used to calculate the coefficients Foster [12] and the process used to prepare the experimental diets [5]. The selection of the most digestible ingredients makes it possible to improve zootechnical indices and reduce water pollution [26].

Other authors state that the low digestibility of vegetable by-products such as passion fruit cake PFC is normally attributed to high levels of non-starch polysaccharides (NSP) Wing [34] or cell wall composition Dusterhoft and Voragen [11]. Wing [34] states that these antinutritional factors reduce the digestibility and uptake of the nutrients in PFC, either by direct encapsulation of the nutrients or by increasing the viscosity of the intestinal content, thus reducing the rate of hydrolysis and absorption of the nutrients in the feed. This could explain the poorer yields found with the 9% diet compared to the control diets. The addition of proteolytic, fibrolytic or carbohydrate degrading enzymes to PFC diets has great potential for releasing unavailable nutrients and energy.

Significant differences ($P \leq 0.05$) were found in PER between all DT and significant differences ($P \leq 0.05$) were found in PVP between the control DT T1, T2 and T4. However, the lowest values were obtained with this later DT (9% PFC).

Regarding the yield of green terror in the fattening stage, the results obtained for the PER and PVP in this study are relatively low compared to the results obtained by Bermúdez *et al*. [7] with Nile tilapia, Yudy *et al*. [35] with yamú (*Brycon siebenthalae*), Madrid [19] with gulf corvina (*Cynoscion othonopterus*) juveniles, and Madrid [19] with sabaleta (*Briycon henni*).

This could be because this native fish has different feeding habits from those of the fish species compared, and the partial replacement of the fishmeal could have reduced the efficiency rates PVP and PER. Moreover, it should be noted that this native fish has not undergone genetic improvement in any physiological stage, and it has not been exploited using intensive or semi-intensive production systems based on balanced feed, that would have allowed its organism to adapt to consuming balanced feed.

When comparing the mortality between trails, it can be seen that it was very low, and fish deaths occurred due to handling during initial and final weighing, measurement of external anatomical dimensions and fish transfer to the metabolic aquariums of the experiment. Mortality can also be attributed to attacks by *Ichthyopthirius* fungi and external parasites. The mortality was therefore not due to the experimental diets (TABLE IV).

Productive performance

The yield of the native species “green terror” fed different isoproteic and isocaloric diets is shown in TABLE IV. After

TABLE IV

EFFECT OF DIETS CONTAINING DIFFERENT PERCENTAGES OF PASSION FRUIT CAKE ON THE PRODUCTIVE PERFORMANCE INDEX OF *Andinoacara rivulatus* IN THE FATTENING PHASE

	Dietary Treatments (DT)			
Variable[1]	T1	T2	T3	T4
Final weight (g)	49.25± 1.10 **a**	49.00±1.10 **a**	47.25±1.10 **b**	45.00± 1.10 **c**
Weight increase (g)	14.25± 1.10 **a**	14.00± 1.10 **a**	12.25± 1.10 **b**	10.00± 1.10 **c**
Relative weight increase (g).	40.70±3.16 **a**	40.00±3.16 **a**	35.00± 3.16 **b**	28.58± 3.160**c**
Growth rate (%)	47.50± 3.68 **a**	46.68±3.68 **a**	40.82± 3.68 **b**	33.35± 3.68 **c**
Incremental growth rate (%)	1.15± 0.08 **a**	1.12± 0.08 **a**	1.00± 0.08 **b**	0.85± 0.086 **c**
Net feed consumption (g)	80.00± 0.80 **a**	78.00±0.80 **b**	76.00± 0.80 **c**	74.00±0.80 **d**
Feed conversion ratio (g)	1.63± 0.02 **a**	1.59± 0.02 **b**	1.61± 0.02 **b**	1.64± 0.02 **a**
Feed cost ($ Kg. $^{-1}$)	0.72± 0.001 **a**	0.71± 0.001 **b**	0.70± 0.001 **c**	0.69± 0.001 **d**
Mortality (%)	5	4	2	3

[abc] Averages with different letters in the same line differ statistically according to the Tukey test

[1] These are the means (± EEM) of 25 fish housed in a cage (Experimental Unit) with four replicates per trial. T1: 0% Passion Fruit Cake, T2: 3% Passion Fruit Cake, T3: 6% passion fruit cake and T4: 9% passion fruit cake.

supplying the experimental diets during a research period of 30 d during the fattening season, no significant differences were found ($P \leq 0.05$) for Wf, WI, RWI, GR and IGR of “green terror”, between DT T1 and T2 when PFC was added; however, these two DT did show differences compared to DT T3 and T4, which showed poorer results than DT T1.

Some initial studies suggest that palm kernel cake can be tolerated even up to 30% in rations for catfish (*Clarias gariepinus*) and 20% for tilapia, well above the levels in this work, without any adverse effects on growth or yield [27].

With the same species, Wan [33] and Wing [34] found satisfactory results up to a level of 20%. The yield did not vary when cachama were fed this alternative source of protein Vasquez [31], so these can also serve as a reference for the use of PFC.

The results do not of this work agree with those obtained with palm kernel cake (PKC) for diets of red tilapia fingerlings, which also incorporated PKC up to a level of 8% without affecting the fish yield, which suggests that higher levels could be used. Other authors have also used PKC as an organic ingredient for the partial or total replacement of fishmeal for aquatic species up to 8% without negative effects on the yield [32].

Net feed consumption exhibited significant differences ($P \leq 0.05$) between DT T1, T2, T3 and T4. The consumption of feed decreased when the PFC in the diets of the fish was increased.

Significant differences in feed conversion ratio were found ($P \leq 0.05$) between DT T1 and T4, in compared to DT T2 and T3, and the best conversions occurred in these DT compared to the control DT.

As with many ingredients of vegetable-based and oil-seed feeds, there are several factors that can limit the inclusion of PFC in fish diets. These include its relatively low protein content, possible amino acid deficiency, and antinutritional factors [33]. However, in the present study, diets with various percentages o PFC used 59% total protein export quality fishmeal, which provided adequate levels of amino acids. Moreover, it was supplemented with nutritional additives including a pre-mix of vitamins and minerals and synthetic methionine.

Therefore, amino acid deficiency does not seem to be the cause of the poorer yields obtained with the diet containing the highest percentage (9%), despite the high percentage of crude fiber that could possibly have had a lower availability of essential amino acids. This native species has not been exploited in captivity nor fed balanced feed, so its digestive system has not adapted to this type of food and did not produce the necessary enzymes for digesting these nutrients (protein, oil, fiber, etc.).

Regarding feed costs, the price per kg of the diet prepared with the highest fishmeal content (0% PFC) was seen to be higher, as its cost was quite high. When the fishmeal in the diet was partially replaced, the cost per kilogram of feed (3, 6 and 9% PFC) was reduced. It should be noted that the addition of amino acid supplements was not necessary in diets containing PFC, so there was no increase in the added cost, which is why it is considered to be a cheaper alternative to fishmeal.

Ng *et al*. [24] in work carried out with tilapia that were fed PFC pre-treated with enzymes for commercial feed, consistently found greater efficiency in growth and feed utilization compared to fish fed similar levels of raw PKC. They then indicated that up to 30% PKC treated with enzymes could be incorporated into the

diet of red tilapia without slowing its growth significantly, which was corroborated by Wing [34]. Therefore, the enzyme addition is key to achieve higher PFC content without negative effects on zootechnnical parameters and performance. That is the reason why an enzymatic complex based on protease, xylanase and amylase was added in our experimental diets.

CONCLUSIONS

Green terror has high capacity to utilize the nutrients in diets prepared with up to 3% PFC, supplemented by an enzymatic complex during fattening, without affecting the yield of fish in terms of final weight, weight increase, relative weight increase, growth rate or incremental growth rate and significantly decreasing the cost of the diet. The diet with 3% PFC obtained the best feed conversion rate. Further, the digestibility coefficients of protein and energy are higher for levels of up to 6% PFC.

However, at increased levels of PFC, the protein efficiency rate and the productive value of protein during the fattening stage are decreased for “green terror”.

The greater availability of PFC throughout the yr at a lower cost compared to other oil products and raw materials such as soybean cake, give it a comparative advantage, since it does not have to compete with the demands of other animal species, which in economic terms would also justify its use.

ACKNOWLEDGEMENTS

This research was supported by the Postgraduate Department of the State Technical University of Quevedo, Ecuador and INIA-FSE.

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THE PRICE ELASTICITY OF THE DEMAND AND REVENUE INCREASE FOR SOME FISHERY PRODUCTS

LA ELASTICIDAD PRECIO DE LA DEMANDA Y EL CAMBIO ANUAL EN EL INGRESO PARA ALGUNOS PRODUCTOS PESQUEROS

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ABSTRACT

The price elasticity of demand (PED) measures the variation of the quantity demanded due to a price variation. A concept closely related to PED is the Revenue Increase (RI) that measure weather the demand is elastic or inelastic. The main goal of this paper was to estimate PED and its impacts on the income and demand of six fishery products from Mexico, such as Salmon, Tuna, Sardine, Shrimp and Prawn, Trout and Tilapia. The data were obtained from the Foreign Agriculture Service of United States Department of Agriculture (1,998-2,018 Period) through the tables provided and published on the Internet (secondary data). In this paper, the arc method was applied to calculate both PED and RI of the selected fishery products. All of these products showed an elastic demand price in almost all years of the period under study; while the RI presented no defined trend. There was a significant positive correlation between export reference price of demand and income for Tuna and significant negative for Trout and Sardine. There was a significant negative correlation between exported volume and export reference price for Shrimp and Prawn, Trout and Sardine and significant positive for Tuna. For Salmon and Tilapia, the associations were not significant. It was observed no clear effects of the PED on income; aspect that violates the PED theory.

Key words: Economics; elasticity; price of demand; sea products; revenue increase

RESUMEN

La elasticidad precio de la demanda (PED en inglés) mide la variación de la cantidad demandada debido a la variación en el precio. Un concepto íntimamente relacionado al PED es el Aumento de los Ingresos (RI en inglés). El objetivo de este trabajo fue estimar la PED y su impacto en los ingresos y la demanda de seis productos pesqueros de México, como Salmón, Atún, Sardina, Camarones y Gambas, Trucha y, Tilapia. Los datos se obtuvieron del Servicio de Agricultura Exterior del Departamento de Agricultura de Estados Unidos (período 1.998-2.018) a través de las tablas proporcionadas y publicadas en Internet (datos secundarios). En este trabajo se aplicó el método de arco para calcular el PED y el RI de los productos seleccionados. Estos seis productos mostraron un precio de demanda relativamente elástico en la mayoría de los años, mientras que el RI mostró una tendencia no definida. Se encontró una correlación positivamente significativa entre el precio de referencia de exportación de la demanda y el ingreso para el Atún, y negativo significativo para la Trucha y la Sardina. Se determinó también una correlación negativamente significativa entre el volumen exportado y el precio de referencia de exportación para Camarones y Gambas, Truchas y Sardinas, y positivo significativo para Atún. Para Salmón y Tilapia, las asociaciones no fueron significativas. No se observaron efectos claros de la PED en los ingresos, aspecto que viola la teoría PED.

Palabras clave: Economía; elasticidad; precio de la demanda; productos del mar; incremento de ingresos

Recibido: 26/02/2020 Aceptado: 15/09/2020

INTRODUCTION

Mexico is currently the third largest merchandise trading partner of United States of America (USA) with $ 611.5 billions (b) in bidirectional trade in goods during 2,018. Exports of goods totaled $ 265.0 b; imports of goods amounted to $ 346.5 b. The USA trade deficit with Mexico was $ 81.5 b in 2,018. Trade in services with Mexico (exports and imports) amounted to an estimated $ 59.4 b in 2,018. Service exports were $ 34.1 b; imports of services were $ 25.3 b. The USA trade services surplus with Mexico was $ 8.8 b in 2,018. Mexico was the second largest supplier of imports of goods from the USA in 2,018.

The top import categories in 2,018 was found: vehicles ($ 93 b), electrical machinery ($ 64 b), machinery ($ 63 b), mineral fuels ($ 16 b), and optical and medical instruments ($ 15 b). Total USA imports of agricultural products from Mexico amounted to $ 26 b in 2,018, the largest supplier of agricultural imports of USA. Main categories include: fresh vegetables ($ 5.9 b), other fresh fruits ($ 5.8 b), wine and beer ($ 3.6 b), snack products ($ 2.2 b), and fruits and processed vegetables ($ 1.7 b). US imports of services from Mexico were an estimated $ 25.3 b in 2,018, 0.6% ($ 164 million (m)) less than 2,017, but 59.3% higher than the levels reported in 2,008.

The law of demand [8] establishes that the existing relationship for a good and the quantity demanded is inverse, so the demand curve is descending (or with a negative slope) and the variables that have the most influence on demand are: the price of the own good, personal income, prices of related goods (substitutes or complementary), tastes and preferences, season, among others. In this sense, the elasticity of a price is usually expressed as a negative number, which represents a positive percentage value. It is from here that elasticity can be understood or defined as the percentage variation of one variable x in relation to another variable y. If the percentage variation of the dependent variable y is greater than the independent variable x, the relationship is said to be elastic, since the dependent variable y varies in greater quantity than that of the variable x.

In contrast, if the percentage variation of the variable x is greater than that of y, the relationship is inelastic. The inelasticity or elasticity of one variable in relation to another reflects, that if it is inelastic, the change in percentage terms made by the independent variable on the dependent is small, however if it is elastic, the percentage variation of the independent variable on the dependent it is notorious. Mathematically, elasticity can be expressed as the proportional change from one variable to another variable. The concept of elasticity can be used as long as there is a cause and effect relationship. In this way, the elasticity of the demand price is the proportional variation of the quantity demanded before a proportional variation of the price [4].

Mexico is the 4th most important fishing Country in America and occupies the 17th place in world fisheries production. Thanks to Mexico having privileged climatic and territorial conditions, a wide variety of crustacean, mollusk and fish can be found. The most representative species for the amount of income they generate in Mexico are: Tuna (*Thunnus* spp.), Mojarra (*Mayaheros urophthalmus*) and Shrimp (*Farfantepenaeus* spp.). Tuna and Shrimp fishing occur in almost all States that have a sea coast. The Mojarra is fished in practically all the national territory because it can be grown in estuaries and in freshwater ponds. Other important fishery products are Sardine (*Sardinops* spp.), Octopus (*Octopus vulgaris*), Lobster (*Panulirus interruptus*), Yellowfin Tuna (*T. albacares*), Bass (*Morone* spp.), Red Snapper (*Lutjanus* spp,) and Oyster (*Crassostrea* spp.), in addition to forty other species with lower production. Fishing in rivers, lakes, lagoons, dams and estuaries is smaller but of great value to some regions of Mexico for their food and economic contribution. In these internal bodies of water, fish or other aquatic organisms such as Trout (*Oncorhynchus* spp.), Bass, Catfish (*Ariopsis* spp.), Shrimp and Prawns (*Litopenaeus* spp.) are usually planted, which are produced through aquaculture [6].

In relation to the aquaculture production in Mexico, it generated a total of 404 thousand Tons (T) of fish and shellfish grown in coastal marine areas, inland waters and ponds in the national territory during 2,017, with a value of 17,813 million of Mexican pesos (Mp), which allowed to reactivate and boost the economy in rural communities of the national territory. Due to its impact on marginalized areas and in many rural communities in Mexico, aquaculture has been a determining factor in overcoming poverty, which is demonstrable by the high impacts and achievements that have been obtained. In addition, it was noted that in 2,013, aquaculture production was 246 thousand T worth seven thousand 568 Mp; However, with the impulse of incentives for the development of this activity and the efforts of thousands of producers throughout the Country, production increased 158 thousand T. Currently, the main aquaculture species in Mexico are Shrimp (150 thousand 76 T); Tilapia Mojarra (149 thousand 54 T); Oyster (45 thousand 148 T), Carp (30 thousand 300 T) and Trout (seven thousand T) [2].

The prupose of this research was to estimate the price elasticity of the demand of fishery products, and to determine the impact on this increase in the income of several fishery goods from Mexico such as Salmon, Tuna, Sardine, shrimp, Prawn, Trout, and Tilapia.

MATERIALS AND METHODS

It was selected six of the major export issues in the fishery exportation industry between USA and Mexico: Salmon, Tuna, Sardine, shrimp, Prawn, Trout, and Tilapia. In order to characterize this market, it was proposed to calculate the price elasticity of the demand (PED) and revenue increase (RI) of Salmon, Tuna, Sardine, Shrimp and Prawn, Trout and Tilapia. For this, it was necessary to obtain the data of exports in dollars and volume in metric Tons (MT) of these six fishery products. These data were

gathered from Foreign Agriculture Service (FAS) data Tables for 1,998-2,018 period [3] published on Internet (scondary data). Using this information, the elasticity matrix was created, which is what will be applied in the study. This elasticity matrix is made based on the reference export price in dollars for each MT and the volume exported in MT.

It is indicated that exports expressed in m of dollars will be considered as the general average price by which these fishery products were attained (since the price at which the fishery goods of export are sold and achieved, it is used to analyse in quantitative terms how the market of a certain good adapts or adjusts to variations in the price of the same accounted for in m of dollars, besides that these prices vary according to a change in the real exchange rate) and the record of T of export will be equal to the average annual amount demanded of these fishery products. Based on this, the estimations were determined according to the formula of the price elasticity of the demand of a good. The price elasticity of demand can be estimated using the Arc Method as follows [1, 9]

$$E_d = \frac{\Delta\%Q}{\Delta\%P} \qquad (1)$$

$$?_{??} = \frac{\frac{\Delta Q}{??1}}{\frac{\Delta P}{??1}} = \frac{??1}{??1} \times \frac{\Delta Q}{\Delta P} = \frac{??1}{??1} \times \frac{??2 - ??1}{??2 - ??1} \qquad (2)$$

I

where: P_1 = Initial price, P_2 = Final price, Q_1= Initial quantity and Q_2 = Final quantity

It must be pointed out that for the use of these formulae it was needed to know the amounts demanded at different prices, with all the other factors at constant consumers [7]. Total income (TI) can be defined as the unit price multiplied by the amount demanded, since this is the amount of income received by any seller in a product, who charges a unit price equal to P, multiplied by the total of units sold, Q. (TI = *P* × *Q*). The revenue increase (RI) can be calculated in both initial and final state, utilizing the equation of the total income formula as follow [10]

$$RI = \frac{??2 \times Q2 ?100}{??1?Q1} - 100 \qquad (3)$$

where: P_2, P_1 Q_2 and Q_1 as above.

The data of exports and volume of the six fishery products were introduced in the Excel software for processing and analysing and to estimate the price elasticity of the demand and revenue increase. Pearson's correlation coefficients were calculated between export reference price and total income and exported volume utilizing years (yr) as the common variable. They were calculated for all six Mexican fishery products when demand was elastic and inelastic. The significance of Pearson´s correlation coefficients was determined at 0.01 or 0.05 level of probability using the Statistical Package for Social Science (SPSS) version 25.0 [5].

RESULTS AND DISCUSSION

In this Section it was showed the principal results, and presented the discussion in the frame of the arc method used to calculate the PED and RI values, for all six fishery products exported to USA from Mexico during the period from 1,998 to 2,018. To guide the discussion, the results were presented separately, as follows.

Salmon:

The price elasticity of demand of Salmon oscillated from 0.63 (yr 2,007) and 22.29 (yr 2,009), the demand shows almost an elastic behaviour in 13 yr (PED>1), with an inelastic behaviour only in 1 yr (PED < 1). Also, the PED=1 in 4 yr, as shown in

As can be seen from TABLE I, the value of the revenue increase was negative in 10 yr and positive in other 10 yr. The highest RI was observed in 2,008 (1,175.57%) with the lowest values in 2,011 and 2,014 (-100.00%). The highest exported quantity was reached in 1,999 (419.0 MT) and the lowest in quantity in 2,007 (0.8 MT). With respect to the export referential price, it can be said that reaches the highest value in the 2,018 (17,916.2 US dollar, US$/MT) and the lowest in 1,999 (2,545.1 US$/MT). The reference price showed no clear trend. It was also noted that there were no exportations in 2,011 and during the period from 2,014 to 2,016 but continued in 2,017 and 2,018. In 2,018, Salmon products showed an elastic demand of 1.22, with a variation of 1% with respect to the export reference price. This fact has affected the demand in Salmon volume in just 1.22%.

Shrimp and Prawn: The results for this item were shown in TABLE II. Shrimp and Prawn has presented a changing demand, since varied from 0.37 in 2,001 to 10.37 in 2,011. There were 14 elastic demands and six inelastic demands. This indicates that the PED was elastics. With respect to the revenue increase, it was negative in 9 yr and positive in 11 yr. The highest value of the RI was 27.81% in 2,011 and the lowest -31.47% in 2,010 (TABLE II).

As can be noted, the exported volume was highest in 2,009 with 41,121.8 MT (the other case when exported volume overcame 40,000 MT was in 2,007 with 40,559.2 MT), with the lowest value occurring in 2,013, with just 18,486.6 MT (the only case when the exported volume was lower than 20,000 MT). In the case of the export reference price, this was highest during 2,014, with 14,893.5 US$/MT, and lowest during 2,009, with 8,082.1 US$/MT. As in the case of Salmon, the export reference price showed no

TABLE I

PRICE ELASTICITY OF THE DEMAND, REVENUE INCREASE AND OTHER ECONOMIC VARIABLES OF SALMON

	Years 1999-2000									
Variable *	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
ERP(US$/MT)	2,545.1	2,639.6	4,059.0	8,292.2	6,241.2	6,703.5	7,781.2	5,730.0	9,568.4	3,139.6
EV (MT)	419.0	249.4	66.1	1.2	3.4	22.3	14.6	1.1	0.8	31.1
PED	3.50	13.92	2.74	2.81	3.39	20.59	2.80	5.66	0.63	1.88
RI (%)	187.87	-38.27	-59.24	-96.29	113.25	604.47	-24.00	-94.45	21.45	1,175.57
	Years 2009-2018									
Variable *	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
ERP(US$/MT)	3,011.7	11,017.2		5,067.6	3,036.6				7,934.2	17,916.2
EV (MT)	11.4	1.0		32.8	12.8				11.7	32.5
PED	22.29	1.47	1.00	1.00	1.75	1.00			1.00	1.22
RI (%)	-64.84	-67.91	-100.00		-76.62	-100.00				527.25

* ERP: Export reference price; EV: Exported volume; PED: Price elasticity of the demand and RI: Revenue increase. ERP and EV are from FAS [6].

TABLE II

PRICE ELASTICITY OF THE DEMAND, REVENUE INCREASE AND OTHER ECONOMIC VARIABLES OF SHRIMP AND PRAWN

	Years 1999-2000									
Variable *	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
ERP(US$/MT)	11,018.8	13,860.3	12,689.6	10,869.1	11,535.4	11,294.5	11,395.0	9,097.7	8,839.3	9,865.1
EV (MT)	35,056.9	29,063.3	30,022.5	24,295.4	25,494.9	29,001.6	28,080.4	35,377.9	40,559.2	34,494.5
PED	0.51	0.82	0.37	1.36	0.81	6.10	3.64	1.03	4.74	1.47
RI (%)	1.03	4.28	-5.42	-30.69	11.37	11.38	-2.31	0.59	11.39	-5.08
	Years 2009-2018									
Variable *	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
ERP(US$/MT)	8,082.1	9,676.7	9,428.3	9,742.5	14,279.1	14,893.5	11,444.1	11,640.8	11,813.1	11,357.3
EV (MT)	41,121.8	23,536.2	30,873.0	26,292.0	18,486.6	20,356.5	27,995.4	25,324.4	28,539.3	24,884.2
PED	0.88	3.03	10.37	4.89	0.92	2.29	1.21	5.88	8.12	3.48
RI (%)	-2.33	-31.47	27.81	-12.00	3.05	14.85	5.67	-7.99	14.36	-16.17

* ERP: Export reference price; EV: Exported volume; PED: Price elasticity of the demand and RI: Revenue increase. ERP and EV are from FAS [6].

clear trend through this yr. For Shrimp and Prawn, in 2,018, PED was elastic with a value of 3.48, this is, the variation of 1% in the export reference price, originate a variation of 3.48% in Shrimp and Prawn demanded volumes.

Tuna: These results were displayed in TABLE III. As can be seen, the PED for Tuna was inelastic during 7 yr and elastic in 13 yr. The highest PED was obtained in 2,012 with a value of 33.79, and a lowest value of 0.09, 2,018. In the case of the RI, this displays negative values in a 10 yr period, with a lowest of -58.27% in 2,018; and other 10 yr positive-values period, with maximum of 359.44% in 2,016. As can be noticed, the export reference price reached a highest value in 2,018 of about 6,076.5 US$/MT, and lowest in 1,999 with a value of about 2,125.0 US$/MT. Exported volume was highest in 2,017 (8,586.2 MT) and lowest in yr 2,001 (1,316.4 MT), with an exported volume lower than 2,000 MT in 2,000 (1,662.2 MT). It is also observed from TABLE III, that Tuna exports in 2,018 were characterized by an

TABLE III

PRICE ELASTICITY OF THE DEMAND, REVENUE INCREASE AND OTHER ECONOMIC VARIABLES OF TUNA

	Years 1999-2000								
Variable *	1999	2001	2002	2003	2004	2005	2006	2007	2008
ERP(US$/MT)	2,125.6	3,813.6	3,518.1	4,754.8	5,336.7	4,779.0	3,427.7	3,853.9	3,336.9
EV (MT)	4,430.2	1,316.4	2,950.4	3,296.4	3,968.7	5,241.7	4,574.4	4,624.9	4,185.3
PED	2.37	29.05	9.50	0.37	1.60	2.51	0.41	0.09	0.69
RI (%)	33.68	-21.43	106.76	51.00	35.13	18.27	-37.41	13.68	-21.64
	Years 2009-2018								
Variable *	2009	2011	2012	2013	2014	2015	2016	2017	2018
ERP(US$/MT)	2,916.4	3,939.2	3,899.8	5,371.5	5,041.6	4,818.2	5,008.2	5,057.6	6,076.5
EV (MT)	4,494.8	4,213.5	5,937.5	4,956.2	7,387.3	6,453.8	7,807.2	8,586.2	8,434.1
PED	0.53	3.61	33.79	0.57	6.22	2.98	4.91	9.68	0.10
RI (%)	-6.14	32.77	39.51	14.97	39.90	-16.51	25.74	11.06	18.02

* ERP: Export reference price; EV: Exported volume; PED: Price elasticity of the demand and RI: Revenue increase. ERP and EV are from [6].

TABLE IV

PRICE ELASTICITY OF THE DEMAND, REVENUE INCREASE AND OTHER ECONOMIC VARIABLES OF TROUT

	Years 1999-2000								
Variable *	1999	2001	2002	2003	2004	2005	2006	2007	2008
ERP(US$/MT)	3,687.6	3,944.7	3,559.8	3,142.6	2,762.5	3,089.0	3,145.4	3,368.7	3,514.2
EV (MT)	96.6	74.2	64.4	80.0	86.9	36.7	56.5	120.3	37.3
PED	1.65	27.47	1.38	1.74	0.64	7.28	23.48	10.53	24.91
RI (%)	12.05	-36.71	-21.68	9.66	-4.51	-52.78	56.76	128.04	-67.65
	Years 2009-2018								
Variable *	2009	2011	2012	2013	2014	2015	2016	2017	2018
ERP(US$/MT)	4,896.6	4,172.9	6,217.3	5,183.5	6,656.7	4,650.7	5,817.7	4,238.5	5,150.5
EV (MT)	51.0	36.5	46.9	5.9	15.5	36.6	26.4	30.4	15.9
PED	0.94	3.74	0.63	8.56	3.61	2.28	1.45	0.45	3.22
RI (%)	90.52	-21.00	91.44	-89.51	237.38	64.97	-9.77	-16.11	-36.44

* ERP: Export reference price; EV: Exported volume; PED: Price elasticity of the demand and RI: Revenue increase. ERP and EV are from FAS [6].

inelastic PED value of about 0.10.

This means that a variation of 1% in the reference price, can only affect the demanded volume by 0.10%.

Trout. The price elasticity of demand of Trout exports show variations in the range 0.06- 27.47, with a 5 yr inelastic period, and a 15 yr elastic period, as can be noted from TABLE IV. The RI values showed an increase from negative values in an 11 yr period (with lowest value of about -89.51% in 2,018), to positive values within a term of 9 yr (with a maximum of 237.38%), as can be seen in TABLE IV. The highest exported quantity of Trout was reached in 2,007, with an amount of the order of 120.3 MT, with a minimum in 2,018 of about 5.9 MT.

Regarding the export reference price, 2,014 appears to be a critical yr in which, reference prices were subjected to variations from a maximum of 6,656.7 US$/MT to a minimum of 2,762.5 US$/MT. It was observed that in 2,018, the Trout exports had a PED of 3.22%, this is, a variation of 1% in export reference prices of Trout exports during this yr, caused a variation of 3.22% in the amount of Trout demanded.

TABLE V

PRICE ELASTICITY OF THE DEMAND, REVENUE INCREASE AND OTHER ECONOMIC VARIABLES

	Years 1999-2000									
Variable *	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
ERP(US$/MT)	2,339.1						5,760.7	4,305.8	7,362.9	
EV (MT)	7.3						0.4	11.5	2.0	
PED	1.62	1.00					1.00	6.45	2.69	1.00
RI (%)	147.63	-100.00						2,048.90	-70.26	-100.00
	Years 2009-2018									
Variable *	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
ERP(US$/MT)			3,538.7	3,092.7	7,738.0	7,132.7	7,606.0	6,179.3	6,572.7	5,342.0
EV (MT)			118.0	5.7	1,610.6	4,057.3	4,241.3	3,308.7	2,952.9	2,903.9
PED			1.00	13.50	2.32	10.61	0.69	1.19	1.84	0.08
RI (%)				-95.78	70,597.33	132.21	11.47	-36.62	-5.07	-20.07

* ERP: Export reference price; EV: Exported volume; PED: Price elasticity of the demand and RI: Revenue increase. ERP and EV are from FAS [6].

TABLE VI

PRICE ELASTICITY OF THE DEMAND, REVENUE INCREASE AND OTHER ECONOMIC VARIABLES OF SARDINE

	Years 1999-2000								
Variable *	1999	2001	2002	2003	2004	2005	2006	2007	2008
ERP(US$/MT)	943.4	703.2	813.9	1,010.7	950.3	965.3	988.9	1,130.1	1,048.3
EV (MT)	3,960.5	5,105.6	4,113.9	3,901.2	3,760.0	2,727.1	3,987.2	3,015.7	3,518.1
PED	1.49	0.94	1.47	0.25	0.60	20.39	15.53	2.08	2.05
RI (%)	-3.04	3.23	-6.74	17.75	-9.37	-26.33	49.78	-13.57	8.22
	Years 2009-2018								
Variable *	2009	2011	2012	2013	2014	2015	2016	2017	2018
ERP(US$/MT)	790.1	671.2	915.9	1,054.7	1,073.6	1,413.1	733.3	679.6	519.5
EV (MT)	5,759.6	2,104.0	1,556.6	973.1	1,094.1	346.9	3,071.3	4,411.5	
PED	1.72	1.14	0.97	3.27	6.61	3.80	2.52	4.71	3.75
RI (%)	23.39	-27.75	0.95	-28.01	14.44	-58.27	359.44	33.12	129.83

* ERP: Export reference price; EV: Exported volume; PED: Price elasticity of the demand and RI: Revenue increase. ERP and EV are from FAS [6].

Tilapia: The results for Tilapia were shown in TABLE V. It was seen that the demand for this product displays an inelastic demand in the period 2,012-2,018, where the value of the PED decreases from 13.50 in 2,012 to 0.08 in 2,018. It was observed that four isolated yr PED=1, so the price elasticity showed no regular trend. It was noticed an important increase of the RI, from -100% up to 70,597.3% in the period from 2,000 to 2,008. The PED could be estimated within the periods 2,001-2,004, and 2,009-2,010 because there were no exportations of Tilapia during these periods. The same applies to the RI in the periods 2,001-2,005, and 2,009-2,011.

The relation exported volume increases from 0.4 MT in 2,006 up to 4,241.3 MT in 2,016. The export reference price also increases from 2,339.14 US$/MT in 2,000 up to 7,737.97 US$/MT. The variation of the amount of Tilapia demanded with respect to the PED was similar to that of Tuna.

Sardine: As it was seen from TABLE VI, the price elasticity of demand displays an elastic behavior during 15 yr and inelastic in 5 yr, with lowest value of o.25 in 2,003, and highest value of 70.41 in 2,010. On the other hand, the RI varies from negative to positive, with lowest value of -58.27% in 2,015, and 359.44% in 2,016.

TABLE VII
PEARSON´S CORRELATION COEFFICIENTS (P) BETWEEN EXPORT REFERENCE PRICE (ERP) WITH EXPORTED VOLUME (EV) AND INCOME (I)

	Salmon	Shrimp& Prawn	Tuna	Trout	Tilapia	Sardine
Salmon	-0.369 & -0.032*					
Shrimp& Prawn		-0.690††† & 0.030				
Tuna			0.538† & 0.745†††			
Trout				-0.607†† & -0.447†		
Tilapia					0.581 & 0.604	
Sardine						-0.655††† & -0.519††

††† Highly significant (P ≤ 0.01).
†† Significant (P ≤ 0.05).
† Significant (P ≤ 0.10).
r´s without †††, †† and † are not significant (P > 0.10).
* First r´s are between ERP and EV and second r´s are between ERP and income.
Number of observations were 13, 14, 13, 15, 8 and 15 for Salmon, Shrimp and Prawn, Tuna, Trout, Tilapia and Sardine, respectively).
Income was calculated as: I = ERP × EV

The export reference price of Sardine increased from 433.97 US$/MT in 2,001 up to 1,413.14 US$/MT, with exportation volumes varying from 346.97 MT to 13,263.0 MT.

A variation of 1% on the export reference price induces a variation of 3.75% on quantity demand.

Pearson´s correlation coefficients

The Pearson´s correlation coefficients of all six fishery products were shown in TABLE VII and VIII. In TABLE VII it was reported the results for yr in which the PED was elastic. There was no significant relationship (*P*>0.10) between the exported volume and reference price for Salmon and Tilapia, However, for Tuna this relationship was positively significant (*P*>0.10), with a directly proportional relation between the two parameters. On the other hand, for shrimp, Prawn, Trout, and Sardine, this relationship was negatively significant ($P \leq 0.01$, $P \leq 0.01$, $P \leq 0.05$, and $P \leq 0.01$, respectively). This means that exported volume and reference price were inversely proportional.

According to the law of demand of Microeconomics [7], if the goods price increase then the quantity exported decrease. In contrast, for Tuna and Tilapia, this law was not accomplished because the relation was directly proportional: as price of a good increase, the quantity demanded of the good also increases; and as the price of a good decrease, the quantity demanded decreases. In short, a higher price typically causes reduced consumption of the good in question, but it can affect the consumption of other goods as well.

shows the Pearson´s correlation coefficients between exported volume and export reference price, for yr in which the PED was inelastic.

It can be noted that the relationship was not significant ($P > 0.10$) between both the exported volume and reference price, for shrimp, Prawn, Trout, and Sardine. However, for Tuna this relationship was positively significant ($P \leq 0.10$). These indicate that, in the case of Tuna, exported volume and reference price were directly proportional, as in the case of an elastic PED. In shrimp, Prawn, Trout, and Sardine, the relation exported volume/ reference price has no defined trend. Due to the lack of data for Salmon and Tilapia, correlation coefficients could not be estimated. These results are indicative that when the PED is inelastic, the product departs from the demand law.

CONCLUSIONS

In this work it was presented a report on the state of the exports regarding fishery commerce between USA and Mexico in between the period 1,998-2,018. For this study it was selected six of the main fishery products: Salmon, Tuna, Trout, shrimp, Prawn, Tilapia, and Sardine. For this study it was employed the arc method to characterize both the price elasticity demand (PED), and the revenue income.

All selected products showed an elastic demand price in almost all yr in the period under study, with short periods of

TABLE VIII

PEARSON´S CORRELATION COEFFICIENTS (P) BETWEEN EXPORT REFERENCE PRICE (ERP) WITH EXPORTED VOLUME (EV) AND INCOME (I)

	Salmon	Shrimp& Prawn	Tuna	Trout	Tilapia	Sardine
Salmon	NE					
Shrimp& Prawn		-0.846† & -0.032 *				
Tuna			0.634 & 0.846†			
Trout				-0.589 & 0,446		
Tilapia					NE	
Sardine						-0.861† & -0.139

† Significant (P ≤ 0.10). r´s without † are not significant (P > 0.10).

* First r´s are between ERP and EV and second r´s are between ERP and income. NE: No estimated

Number of observations were 1, 6, 7, 5, 2 and 5 for Salmon, Shrimp and Prawn, Tuna, Trout, Tilapia and Sardine, respectively) when elasticity was inelastic. Income was calculated as: I = ERP × EV

inelastic demand. This was in contrast to the revenue increase, which behavior presented no define trend. In periods when the PED was elastic, the relation export volume/export reference price was inversely proportional for shrimp, Prawn, Trout, and Sardine, in agreement with the law of demand. In the case of Tuna and Tilapia, this relation was directly proportional. This may be an indicative of distortions in the market of these products in periods of elastic PED. For periods of inelastic PED, almost all markets depart from the law of demand, or there was no a defined relationship; aspect that violates the PED theory.

Since USA/Mexico is one of the most important commercial partnerships in North America, the results of this work appear to be a very useful tool to predict future trends in fishery exportations between these two Countries.

ACKNOWLEDGEMENT

The authors thank the University of Guanajuato for promoting scientific research as a strategic pillar to strengthen teaching.

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TORTA DE SACHA INCHI *(Pluketenia volubilis)* SUSTITUTO PARCIAL DE SOYA PARA ALIMENTAR POLLOS BROILER

SACHA INCHI CAKE *(Pluketenia volubilis)* PARTIAL SUBSTITUTE FOR SOYA TO FEED BROILER CHICKENS

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RESUMEN

El objetivo de la presente investigación fue estudiar el comportamiento productivo de pollos de engorde que consumen torta de Sacha inchi (TSI) (*Plukenetia volubilis*) como sustituto parcial de la soya (*Glycine max*). Se utilizaron 240 pollos de engorde bebe, de unas horas de nacido, todos machos, de la línea Cobb 500 que fueron alojados según diseño experimental completamente aleatorizado en cuatro tratamientos: testigo (sin TSI y tres niveles (10-20-30%) de inclusión de la misma. Se emplearon 24 unidades experimentales y en cada una se alojaron 10 animales. Se elaboró alimento para las diferentes etapas de desarrollo (Inicial, crecimiento, acabado). El 10% de inclusión de TSI, fue el tratamiento que mostró una mayor ganancia de peso (GP) (P<0,05) con un promedio de 2.666 gramos, mientras que el tratamiento con el 30% de inclusión de TSI, logró el mayor índice de conversión (IC) (P<0,05) con promedio de 1,74. Se evidenció que la TSI puede ser alimento sustituto parcial de la soya para engorde de pollos broilers Cobb 500. Quedó demostrado además, a través del análisis financiero, que todos los tratamientos fueron rentables, sobresaliendo el tratamiento 10% de inclusión de TSI (P<0,05), como el más adecuado.

Palabras clave: Alimento; aves; nutrición; torta; zootecnia

ABSTRACT

The objective of this research was to study the productive behavior of broilers that consume Sacha inchi cake (SIC) (*Plukenetia volubilis*) as a partial substitute for soy (*Glycine max*). Two hundred forty broilers were used, all males of the Cobb 500 line that were housed according to a completely randomized experimental design in four treatments: control (without SIC) and three levels (10-20-30%) of inclusion of the same. Twenty-four experimental units were used and 10 animals were housed in each. Food was prepared for the different stages of development (Initial, growth or fattening, final or finishing). The 10% inclusion of SIC, was the treatment that showed the greatest weight gain (WG) with an average of 2,666 grams, while the treatment with the 30% inclusion of SIC, achieved the highest conversion rate (CRI)with an average of 1,74. It was evidenced that the SIC can be a partial substitute food for broiler broiler cobb 500 soybeans. It was also demonstrated, through the financial analysis that all the treatments were profitable, highlighting the 10% cake inclusion treatment SIC, as the most suitable.

Key words: Food; birds; nutrition; cake; zootechnics

Recibido: 12/02/2020 Aceptado: 24/07/2020

INTRODUCCIÓN

La avicultura se incrementó en las últimas décadas, debido a que el consumo de carne de pollo (*Gallus gallus domesicus*) y de huevo se convirtió en una alternativa para la seguridad alimentaria [15]. A nivel mundial, la carne de ave es la segunda en importancia en volumen de producción, luego de la carne de cerdo (*Sus scrofa domesticus*), es así que en las últimas décadas la tendencia mundial en la demanda de carne blanca aumentó [22]. El pollo de engorde corresponde a las aves criadas para ser engordadas y servir de alimento para el consumo humano. El pollo de engorde moderno se caracteriza por la ganancia de peso (GDP) rápido y uso eficiente de nutrientes [15]. Estos pollos se deben alimentar con vitaminas y proteínas para que al final tengan el peso adecuado para su sacrificio, además de cumplir los parámetros de bioseguridad, esquemas de vacunación, control de desechos y demás medidas de manejo que ayudan al bienestar del animal y por mayor GDP [17]. La producción de pollo de engorde se ha desarrollado y difundido a gran nivel en todos los climas y regiones, debido a su alta adaptabilidad, rentabilidad, aceptación en el mercado y disponibilidad de pollitos de excelente comportamiento productivo y conversión alimenticia [3].

En el Ecuador existe variedad y géneros de frutas y vegetales que contienen gran valor nutricional, al ser un país con gran impulso agrícola; sin embargo, su población desconoce el valor nutricional de sus productos agrícolas, sus potenciales nutricionales y sobre todo como utilizarlos en la alimentación y en especial para las aves de engorde, sin que los mismos pierdan sus nutrientes [24].

El desarrollo de alimentos balanceados con un alto contenido nutricional y de buena calidad, constituye uno de los fundamentos importantes para el desarrollo sostenible de la producción avícola [20]. Lamentablemente la disponibilidad y rendimientos en el cultivo de soya (*Glycine max*), uno de los macro ingredientes en estas raciones, están debajo de la demanda nacional, por lo cual se recurre a la importación de esta materia prima. Esto afecta a los productores avícolas ya que encarece los precios de los insumos, dejándoles sin alternativa alguna al momento de adquirir este insumo y esto hace que compren el balanceado ya elaborado [29]. Debido a esta situación, se encarecen y provocan que los avicultores vean la explotación de aves de corral como una labor no rentable.

La dificultad de la valoración y reconocimiento del activo biológico que presentan las granjas avícolas en Ecuador, radica en poder medir a cada una de las etapas de crianza y engorde para determinar un costo de producción razonable que garantice una mayor rentabilidad en la actividad [10]. Considerando que los productores avícolas buscan la mayor eficiencia posible al menor tiempo [6]. Debido al crecimiento acelerado que tienen las líneas modernas de pollos de carne, causado por el mejoramiento genético, los pollos actuales alcanzan su peso comercial a una edad cada vez más temprana. Esto derivó en que los requerimientos nutricionales se ajusten más a las exigencias que esto demanda. Los requerimientos nutricionales de las aves varían en función al promedio de crecimiento determinado por la edad, factores ambientales y al genotipo [8].

Esto obligó a los investigadores a buscar alternativas de alimentación que cumplan con las composiciones nutricionales que los pollos necesitan para su desarrollo. La presencia en expansión en el país del cultivo de Sacha inchi (*Plukenetia volubilis*) por su interés de la industria en obtener el aceite de esta oleaginosa, presenta alrededor de 48-50% de aceite y 27-28% de proteínas altamente digeribles y ricas en aminoácidos esenciales, excepto leucina y lisina [14]. Los ácidos grasos omega se encuentran dentro de los denominados como esenciales por la razón de que el propio cuerpo humano no lo produce [25]. El aceite obtenido de su semilla está compuesto, en su mayor parte ácidos grasos insaturados (93%) de gran importancia para la nutrición por su alto contenido de ácidos grasos esenciales [19].

La torta es el subproducto resultante del proceso de trituración de las semillas, la misma que se la obtiene con la ayuda de una prensa cilindro extractor de marca Oil Press, semiautomático, modelo AW003, fabricada en Shanghai, China, el aceite comestible y representa una importante fuente de proteínas, especialmente por las características nutricionales y funcionales que puede aportar a los alimentos. El sistema de fabricación que se emplea para facilitar la separación del aceite en las semillas, requiere el uso del vapor indirecto (alta temperatura) y la fricción de la prensa continua durante el proceso [13]. Es importante manifestar que esta torta, presenta una composición química y física aceptable dada por su elevado contenido de proteína y bajo de fibras así como características físicas apropiadas para que pueda ser utilizada en la alimentación animal [1]. La torta la definen como un subproducto resultante de la compresión de las semillas para obtener aceite comestible, lo que es una valiosa fuente de proteínas, por las características nutricionales y funcionales [27].

Teniendo en cuenta lo anterior, el objetivo de este trabajo fue estudiar el comportamiento productivo de pollos de engorde que consumen TSI como sustituto parcial de la soya.

MATERIALES Y MÉTODOS

***Ubicación*.** La investigación fue realizada en los meses de junio a agosto del 2.018 en la Granja Experimental ANDIL-UNESUM ubicada en el Cantón Jipijapa al sur de la provincia de Manabí, Ecuador. Con coordenadas Norte 1º 15' 54" LS y 80º 41' 24" LO, temperatura media anual de 24 a 26 **ºC**, precipitación promedio anual de 250 – 500 milímetros (mm) año (a) y humedad relativa de 60 a 70%. Altura de 0 a 300 metros sobre el nivel del mar (m.s.n.m), pH del suelo de 6 a 7, topografía irregular y textura limoso – arcilloso.

Tratamientos. Se utilizaron 240 pollos de engorde todos machos de la línea Cobb 500 los pollitos bb tuvieron un peso inicial de 42 gramos (g) [7], los mismos se criaron en piso

utilizando una cama de tamo - cáscara de arroz (*Oryza sativa*). Su recibimiento estuvo acorde a los procesos técnicos que se debe realizar en la crianza de esta ave, brindándoles alimento y agua a voluntad al animal. A partir del día (d) siete se distribuyeron en 24 compartimientos o unidades experimentales (UP), donde se alojaron 10 animales UP con una dimensión de 2 x 1 metro (m), ubicando un comedero y un bebedero (Petking) de fabricación China.

Los tratamientos fueron: Control, 10; 20 y 30% de TSI, para sustituir parcialmente la cantidad de soya en la ración se formuló de acuerdo a los requerimientos nutricionales de las aves descritos por la National Research Council (NRC). Los tratamientos fueron aplicados en tres etapas: Al inicio, al crecimiento engorde y al final o acabado (TABLAS I, II y III). TSI se adquirió de la planta industrial de la empresa Omega, ubicada en el cantón San Vicente en la provincia de Manabí. El valor nutritivo de la TSI utilizado en el experimento, es el que se describe a continuación en la TABLA I, reportado por Alcivar y col. [1].

TABLA I
CARACTERIZACIÓN FÍSICA Y QUÍMICA DE LA TSI

INDICADORES (% MS)	MEDIA	DE ±	MÍNIMO	MÁXIMO
MS	89,24	0,40	88,63	89,68
EE	9,05	1,06	7,94	10,68
CENIZA	4,89	0,21	4,56	5,08
PB	41,49	0,35	40,90	41,80
ELN	6,46	1,46	4,97	8,47
FB	7,63	1,77	5,83	10,27
FDN	16,64	1,54	15,32	18,41
FDA	12,70	1,96	9,95	15,03
LIGNINA	1,25	0,20	1,03	1,50
HEMICELULOSA	11,45	1,85	8,70	13,53
Solubilidad (%)	7,96	0,94	6,99	9,18
Volumen (mL/g)	3,92	0,20	3,72	4,22
Capacidad de adsorción de agua (g/g)	2,16	0,49	1,30	2,50

TABLA II
DIETAS EXPERIMENTALES CORRESPONDIENTES AL PERIODO INICIO (0 DÍAS A 10 DÍAS DE EDAD)

Ingredientes %	Control	10% TSI	20% TSI	30% TSI
Coccidiostato	0,05	0,05	0,05	0,05
Anti-hongos	0,05	0,05	0,05	0,05
Antimicotoxinas	0,05	0,05	0,05	0,05
BHT	0,02	0,02	0,02	0,02
Biofos	0,50	0,50	0,50	0,50
Aceite Palma	0,10	0,10	0,10	0,10
Harina de pescado	3,50	3,50	3,50	3,50
Carbonato Ca	1,08	1,08	1,08	1,08
Torta de soja	33,0	29,70	27,4	24,10
Lisina sintética	0,10	0,10	0,10	0,10
Maíz molido	61,00	61,00	60,00	60,00
DL METEONINA 99%	0,15	0,15	0,15	0,15
Pre- mezcla, mineral vitamina	0,10	0,10	0,10	0,10
Polvillo de arroz	0,30	0,30	0,30	0,30
Sacha inchi	0,00	3,30	6,60	9,90
Total	100%	100%	100%	100%
Composición[2] (%)				
PB	22,18	22,05	22,30	22,17
EM (Kcal/Kg)	3101	3132	3154	3180
MS	88,20	88,13	88,19	88, 23
P	0,40	0,41	0,39	0,43
Ca	0,71	0,76	0,78	0,75

1 Porporción 1:1 de: Suplemento vitamínico: vitam. A, 10 000UI; vitam. D3, 2 000 UI; vitam. E, 10 mg; vitam. K, 2 mg; tiamina, 1 mg; riboflavina, 5 mg; piridoxina, 2 mg; vitam. B12, 15.4 µg; ácido nicotínico, 125 mg; pantotenato de calcio, 10 mg; ácido fólico, 0,25 mg; biotina, 0,02 mg.

Suplemento mineral: selenio, 0,1 mg; hierro, 40 mg; cobre, 12 mg; zinc, 120 mg; magnesio, 100 mg; iodo, 2,5 mg; cobalto 0,75 mg. 2 Datos expresados en base seca.

TABLA II

DIETAS EXPERIMENTALES CORRESPONDIENTES AL PERIODO CRECIMIENTO ENGORDE (11 A 22 DÍAS DE EDAD)

Ingredientes %	Control	10% TSI	20% TSI	30% TSI
Coccidiostato	0,05	0,05	0,05	0,05
Anti-hongos	0,05	0,05	0,05	0,05
Antimicotoxinas	0,05	0,05	0,05	0,05
BHT	0,20	0,20	0,20	0,20
Biofos	2,00	2,00	2,00	2,00
Aceite Palma	0,10	0,10	0,10	0,10
Harina de pescado	3,50	3,50	3,50	3,50
Carbonato Ca	1,00	1,00	1,00	1,00
Torta de soja	26,80	24,9	23,98	22,0
Lisina sintética	0,30	0,30	0,30	0,30
Maíz molido	64,46	63,72	62,00	61,34
DL METEONINA 99%	0,24	0,24	0,24	0,24
Pre- mezcla, mineral vitamina	0,25	0,25	0,25	0,25
Polvillo de arroz	1,00	1,00	1,00	1,00
Sacha inchi	0	2,64	5,28	7,92
Total	100%	100%	100%	100%
Composición[2] (%)				
PB	20,04	20,21	20,75	20,89
EM (Kcal/Kg)	3104	2122	3129	3148
MS	87,67	85,22	87,75	87,82
P	0,40	0,44	0,42	0,42
Ca	0,65	0,88	0,87	0,87

1 Porporción 1:1 de: Suplemento vitamínico: vitam. A, 10 000UI; vitam. D3, 2 000 UI; vitam. E, 10 mg; vitam. K, 2 mg; tiamina, 1 mg; riboflavina, 5 mg; piridoxina, 2 mg; vitam. B12, 15.4 µg; ácido nicotínico, 125 mg; pantotenato de calcio, 10 mg; ácido fólico, 0,25 mg; biotina, 0,02 mg.

Suplemento mineral: selenio, 0,1 mg; hierro, 40 mg; cobre, 12 mg; zinc, 120 mg; magnesio, 100 mg; iodo, 2,5 mg; cobalto 0,75 mg. 2 Datos expresados en base seca.

TABLA III

DIETAS EXPERIMENTALES CORRESPONDIENTES AL PERIODO FINAL O ACABADO (23 A 42 DÍAS DE EDAD)

Ingredientes %	Control	10%TSI	20% TSI	30% TSI
Coccidiostato	0,05	0,05	0,05	0,05
Anti-hongos	0,05	0,05	0,05	0,05
Antimicotoxinas	0,05	0,05	0,05	0,05
BHT	0,02	0,02	0,02	0,02
Biofos	2,20	2,20	2,20	2,20
Aceite Palma	0,10	0,10	0,10	0,10
Harina de pescado	0,00	0,00	0,00	0,00
Carbonato Ca	1,10	1,10	1,10	1,10
Torta de soja	26,00	23,40	21,00	18,60
Lisina sintética	0,97	0,97	0,97	0,97
Maíz molido	62,00	62,00	61,80	61,60
DL METEONINA 99%	0,20	0,20	0,20	0,20
Pre- mezcla	0,26	0,26	0,26	0,26
Polvillo de arroz	7,00	7,00	7,00	7,00
Sacha inchi	0,00	2,60	5,20	7,80
Total	100%	100%	100%	100%
Composición[2] (%)				
PB	18,98	18,88	18,86	18,83
EM (Kcal/Kg)	3107	3133	3156	3179
MS	84,65	86.28	84,82	85,16
P	0,36	0,41	0,41	0,41
Ca	0,76	0,72	0,79	0,79

1 Proporción 1:1 de: Suplemento vitamínico: vitam. A, 10 000UI; vitam. D3, 2 000 UI; vitam. E, 10 mg; vitam. K, 2 mg; tiamina, 1 mg; riboflavina, 5 mg; piridoxina, 2 mg; vitam. B12, 15,4 µg; ácido nicotínico, 125 mg; pantotenato de calcio, 10 mg; ácido fólico, 0,25 mg; biotina, 0,02 mg. Suplemento mineral: selenio, 0,1 mg; hierro, 40 mg; cobre, 12 mg; zinc, 120 mg; magnesio, 100 mg; iodo, 2,5 mg; cobalto 0,75 mg. 2. Datos expresados en base seca.

Manejo de la investigación.

Los siete primeros d, los animales se adaptaron al medio ambiente del galpón, posteriormente se ubicaron en los compartimientos ya mencionados, se aplicó la distribución aleatoria según el diseño experimental, se ubicó alimento según los niveles de inclusión de TSI y agua a los animales. Se consideró en todo momento el consumo de alimento expuesto en la tabla

de rendimiento y nutrición del híbrido Cobb 500, el cual indica el promedio de alimento que según el pollo debe de comer. Con la ayuda de balanza digital, Cas PRPLUS – 30, (fabricación China), se pesó el alimento, se controló el rechazo por tratamiento, se llevó el control diario y semanal. Se pesaron los animales con la finalidad de obtener los parámetros, GDP y CoA además, mediante tablas de registros se controló la mortalidad entre tratamientos.

Variables de respuesta

Conversión alimenticia (CoA) (gramos - g). Se obtuvo pesando semanalmente todos y cada uno de los pollos y la alimentación diaria.

Consumo de alimento diario (CAD) (g). Esta variable se calculó dividiendo el consumo entre el número promedio de aves evaluadas en la semana.

Ganancia de peso acumulado (GPA) (g). Se determinó haciendo la diferencia entre la semana anterior menos la posterior hasta la sexta semana.

Mortalidad (M) (%). Esta variable se obtuvo contando la cantidad de aves muertas durante la investigación.

Análisis económico

Para determinar el beneficio/costo de los tratamientos se realizó un análisis de presupuestos parciales [6].

Análisis estadístico

Se utilizó un diseño completamente aleatorizado con cuatro tratamientos que consistieron en las dietas experimentales. Para el análisis de los resultados se utilizó el paquete estadístico computarizado INFOSTAT [12]. Para la comparación media de medios se utilizó la prueba múltiple de Duncan al P<0,05 de probabilidad. [16].

RESULTADOS Y DISUSION

Consumo de alimento (CA)

La TABLA IV hace referencia al CA de los pollos hasta el d 42 de crianza. Se observó que en todos los tratamientos, los animales consumieron las dietas experimentales según las normas técnicas recomendadas por Cobb 500 [9] y se observó una ligera tendencia al aumento en los tratamientos que incluyeron la TSI.

TABLA IV
CONSUMO DE ALIMENTO AL DÍA 42 DE POLLOS QUE CONSUMEN DIFERENTES NIVELES DE TORTA DE SACHA INCHI

Tratamientos	Consumo alimento (g/ave)	Cantidad (aves/ tratamiento)	Consumo alimento (kg/ tratamiento)
Testigo	4.139	60	248,3
10%T.S.I	4.486	58	269,2
20%T.S.I	4.341	58	260,5
30%T.S.I	4.271	58	256,3

En la TABLA V se muestran los resultados productivos de las diferentes variables estudiadas. Se observó mayor peso vivo y ganancia (P<0,05) en el periodo de 0 a 42 d para el tratamiento que incluyó el 10% de TSI con respecto al control y el resto de los tratamientos que no difirieron entre sí (P<0,05). No se observaron diferencias (P<0,05) para la CA en la etapa.

Veloz [30] utilizó harina de Sacha inchi en la crianza de pollos broiler, reportando un CA al d 49 de crianza de 2.892,88 g. Por otra parte, Muirragui [21] obtuvo un CA promedio de 3.148 g. Estos CA fueron inferiores a los encontrados en el presente estudio, para todos los tratamientos. Las causas de las diferencias pueden ser diversas. Ambos autores emplearon el ingrediente en forma de harina la cual difiere en composición química con respecto a la torta. Por otra parte, el sistema de manejo que se empleó fue diferente, utilizando jaulas, a diferencia del presente en el que se utilizó en piso con cama de tamo de arroz. Las variaciones ambientales también pudieron haber influido como factores extrínsecos, correspondientes al manejo.

Ganancia de peso (GP)

Tomando como referencia los resultados, obtenido en la investigación y la discusión de los mismos con otros autores, se puede destacar que, con el CA se logró ganar mayor GP, dejando reflejado que no existen inconvenientes al momento del consumo voluntario de los pollos, destacando que en algunas investigaciones se ha utilizado la torta, harina, aceite de Sacha inchi, en distintos niveles de inclusión en la dieta.

TABLA V
RESULTADOS PRODUCTIVOS DE POLLOS DE CEBA QUE CONSUMEN DIFERENTES NIVELES DE TORTA DE SACHA INCHI

Tratamientos	Control	10% TSI	20% TSI	30% TSI	EE± Significación
Peso inicio (g)	*41,70*	41,70	*41,63*	*41,83*	0,16
Peso 42 días (g)	*2475*[b]	*2708*[a]	*2552*[b]	*2513*[b]	37,39 **
Conversión 0-42d	1,71	1,69	1,73	1,74	0,03
Ganancia 0-42d (g)	*2433*[b]	*2667*[a]	*2511*[b]	*2472*[b]	37,36 **

Medias con letras distintas son significativas para P<0,05 (Duncan, 1955) Altamente significativo: **P<0,01

Los resultados encontrados en la presente investigación están por encima de los reportados por Reatigui [26], quien encontró que la GP a los 45 d en los pollos parrilleros fue de 2.144 g, cuando utilizó 40% de TSI. Veloz [30], al utilizar harina de Sacha inchi en la crianza de pollos broiler, reportó GP de 1.601,25 g a los 49 d. Así mismo, Ramírez [23] utilizó el 25 y 50% de TSI sustituyendo la soya en la ración, y obtuvo a los 45 d de edad de los pollos, una GP de 1.576,8 y 1.352,7 g, respectivamente.

Reátegui [25], observó que los pollos alimentados con raciones incluidas de 0 y 7% de TSI, consumieron más alimento en relación a los pollos alimentados con 14% de TSI. Asimismo, encontró que hubo mayor GP a mayor inclusión de torta. Este resultado, concuerdan con lo reportado por Tang y Capuñay [20], quienes obtuvieron mayores GP con el 10% de inclusión de TSI en la ración; por lo tanto estos dos últimos resultados coinciden con la presente investigación.

En referencia a la CA, Ramírez [23], encontró un índice superior a 2. Tang y Capuñay [20] obtuvieron rangos de CA entre el 1,9 a 2,01. Veloz [21], al utilizar TSI en la crianza de pollos broiler, reportó una CA de 1,45.

La utilización de 0;2; 4 y 6% de Sacha inchi en la alimentación de pollos broilers, permitió registrar una CA a los 29 a 49 d de 2,17; 2,18; 2,17 y 2,18 respectivamente, [2].

Tang y Capuñan [28] manifiestan que la CA reflejó mejores resultados para los tratamientos donde se incluyeron los mayores porcentajes de TSI, el autor manifiesta, qué el pollo de engorde ha sido genéticamente desarrollado para que gane peso extremadamente rápido y usando eficientemente los nutrientes. Por ello es tan indispensable el manejo correcto a los pollos de hoy en d, ellos consistentemente tendrán gran eficiencia y economía. Se evidencia que el 10% ganaron mayor peso, pero es necesario manifestar que los demás tratamientos también generaron ganancia de peso.

Mortalidad (M)

Con respecto a la M no se reportó en las primeras dos semanas (sem). Posteriormente, en la tercera sem con el 20% de T.S.I, murió un pollo, al igual que en la cuarta y la quinta sem con el tratamiento del 30% de T.S.I. En la sexta sem fallecieron dos animales con el control y uno con el 10% de TSI.

Se debe resaltar que el índice de mortalidad se consideró bajo, ya que la población fue de 240 pollos, de los cuales seis pollos murieron en el transcurso del experimento, lo que corresponde al 2,5% de (M), aspecto que concuerda con Muirragui [21], quién no registró M en la etapa de crecimiento, las aves finalizaron con un estado sanitario satisfactorio, esto puede deberse a que la TSI contiene vitaminas con capacidad antioxidante, así también es rica en aceites OMEGA 3 y 6 que ayudan a incrementar el sistema inmunológico de los animales [11].

De acuerdo con Chirinos [11] y Henao y Barreto [18], al analizar los ácidos grasos poliinsaturados, tocoferoles, fitosteroles, compuestos fenólicos y la capacidad antioxidante de la semilla de 16 diferentes variedades de sacha inchi, encontraron significativas diferencias en los contenidos de aceites OMEGAS 3 y 6, su capacidad antioxidante y otros compuestos fitoquímicos, concluyendo que su consumo puede ser considerado como una importante fuente de promotores fitoquímicos saludables.

Análisis económico

En la TABLA VI se detalla la evaluación económica de los tratamientos. El mismo muestra el análisis Beneficio/Costo. Se observó que todos los tratamientos que incluyeron Sacha inchi, mostraron mejoras económicas respecto al control y el 10 % fue el que sobresalió entre ellos para todos los indicadores estudiados.

TABLA VI
EVALUACIÓN ECONÓMICA DEL USO DE DIFERENTES NIVELES DE TORTA DE SACHA INCHI PARA LA CRIANZA DE POLLOS DE ENGORDE

Indicadores	Tratamientos			
	Control	10% T.S.I	20% T.S.I	30% T.S.I
Rendimiento de carne en Kg (B)	148,64	162,55	153,27	150,82
Precio Unitario Kg (C)	2,20	2,20	2,20	2,20
Ingreso Bruto Total (USD)	327,00	357,60	337,20	331,80
Utilidad Neta Total (USD)	143,01	173,34	157,20	153,84
Relación: Beneficio / Costo (B/C)	1,78	1,94	1,87	1,86
Rentabilidad (%)	77,73	94,07	87,33	86,44
Costo de producción por unidad (USD/Kg)	1,24	1,13	1,17	1,18

B: Beneficio, C: Costo

El análisis económico pude evidenciar que el 10 % de inclusión de TSI obtuvo mejores resultados con un ingreso de 357,60 dólares, por la cantidad de kilogramos vendidos, con referencia a los demás tratamientos de esta investigación; con la inclusión del 10 % de TSI, en la ración se obtuvo la más alta relación beneficio con un 1,94. Tang y Capuñay [28] no obtuvieron mayor beneficio económico en su experimento, así tampoco Muirragui [21], obtuvo un valor por debajo de los obtenidos en la presente investigación.

Los valores obtenidos demuestran que el uso de la TSI se la puede utilizar como fuente proteica y brinda buenos resultados económicos.

CONCLUSIONES

Se evidenció que la TSI puede ser alimento sustituto parcial de la soya para engorde de pollos broiler Cobb 500. Quedó demostrado, además, a través del análisis financiero, que todos los tratamientos fueron rentables, sobresaliendo el tratamiento 10% de inclusión de TSI, como el más adecuado.

AGRADECIMIENTOS

A la Universidad Estatal del Sur de Manabí (UNESUM) que contribuyó para la realización de esta investigación, que forma parte del proyecto titulado " Torta de sacha inchi (*Plukenetia volubilis*) como fuente de proteínas en pollos de engorde". Al Instituto de Ciencia Animal de la República de Cuba, por su contribución técnica, profesional y de formación doctoral del primer autor.

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DESCRIPTION OF HUTMANNIN-1, A NEW PIII-METALLOPROTEASE FROM THE VENOM OF THE NEOTROPICAL LANSBERG'S HOGNOSE VIPER *(Porthidium lansbergii hutmanni)* WITH FIBRINO(GENO)LYTIC AND HAEMORRHAGIC ACTIVITIES

DESCRIPCIÓN DE HUTMANNIN-1, UNA NUEVA METALOPROTEASA PIII DEL VENENO DE LA SERPIENTE NEOTROPICAL MAPANARE DE LANSBERG *(Porthidium lansbergii hutmanni)* CON ACTIVIDADES FIBRINO (GENO) LÍTICAS Y HEMORRÁGICAS

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ABSTRACT

The objective was to characterise hutmannin-1 (hut-1), a new ~ 62 kDa P-III-class metalloprotease from *Porthidium lansbergii hutmanni* (*P.l.h*) (Margarita Island, Venezuela). To characterise this protein, the crude venom of *P.l.h* was fractionated by size exclusion chromatography, anion exchange chromatography and High Performance Liquid Chromatography (HPLC). Hutmannin-1 was identified by MALDI-TOF/TOF mass spectrometry, and the venom was analysed by SDS-PAGE. The lethality, minimum haemorrhagic dose (MHD), effect of temperature on the activity, procoagulant activity on human plasma, and anticoagulant, defibrinating, gelatinolytic fibrinolytic, and fibrinogenolytic and platelet aggregation activities of hut-1 were determined. Antigenic recognition assays were performed on *P.l.h* crude venom and hut-1 by a venezuelan polyvalent anti-ophidic serum (PAOS) Hut-1 had strong fibrinogenolytic and moderate fibrinolytic activity. These activities and the haemorrhagic activity of hut-1 were completely inhibited by EDTA. P.l.h crude venom had potent anticoagulant activity on recalcified plasma and inhibited the platelet aggregation induced by thrombin, ADP, collagen and ristocetin. In contrast, the anticoagulant, coagulant and platelet aggregation inhibition of hut-1 were not observed with any of the agonists. This result suggests that other proteins in the crude venom, markedly impact platelet functions and/or coagulation factors. Commercial venezuelan antivenin showed limited ability to neutralise the haemorrhagic activity of hut-1.

Key words: Anticoagulant; antivenin; coagulation; haemostasis; haemorrhage; snake venom

RESUMEN

El objetivo de este trabajo fue caracterizar hutmannin-1 (hut-1), una nueva metaloproteasa de clase P-III de ~ 62 kDa de la serpiente *Porthidium lansbergii hutmanni* (*P.l.h*) (Isla Margarita, Venezuela). Para caracterizar esta proteína, el veneno crudo de *P.l.h* se fraccionó mediante cromatografía de exclusión molecular, cromatografía de intercambio aniónico y cromatografía líquida del alto rendimiento (HPLC). Hutmannin-1 se identificó por espectrometría de masas MALDI-TOF / TOF, y el veneno se analizó por SDS-PAGE. Se determinó la letalidad, la dosis hemorrágica mínima (MHD), el efecto de la temperatura sobre la actividad, la actividad procoagulante en el plasma humano y las actividades anticoagulantes, desfibrinantes, fibrinolíticos gelatinolíticos y fibrinogenolíticos y de agregación plaquetaria de hut-1. Se realizaron ensayos de reconocimiento antigénico en *P.l.h* veneno crudo y hut-1 mediante suero anti-ofídico polivalente (PAOS) venezolano. Hut-1 tuvo una fuerte actividad fibrinolítica y fibrinolítica moderada. Estas actividades y la actividad hemorrágica de hut-1 fueron completamente inhibidas por ethylenediaminetetraacetic acid (EDTA). El veneno crudo de *P.l.h* tuvo una potente actividad anticoagulante en plasma recalcificado e inhibió la agregación plaquetaria inducida por trombina, ADP, colágeno y ristocetina. En contraste, la inhibición de la agregación plaquetaria, actividad anticoagulante y coagulante de hut-1 no se observó con ninguno de los agonistas. Este resultado sugiere que otras proteínas en el veneno crudo, afectan notablemente las funciones plaquetarias y / o los factores de coagulación. El antiveneno comercial venezolano mostró una capacidad limitada para neutralizar la actividad hemorrágica de la hut-1.

Palabras clave: Anticoagulante; antitoxina; coagulación; hemostasia; hemorragia; veneno de serpiente

Recibido: 24/01/2020 Aceptado: 12/08/2020

INTRODUCTION

Snake bite is a significant work-related and countryside menace in the tropical and sub-tropical Countries. Precise statistics of the occurrence of snakebite and its morbidity and mortality throughout different geographical areas does not exist; nevertheless, it is sure that it is higher than what is officially reported. Clinical and toxinologically, description of snake envenomations are considered into haemotoxic, neurotoxic, and myotoxic pathological conditions.

Haemorrhagic signs are the characteristic symptoms associated with Viperidae snake bites. This activity has been attributed to haemorrhagic enzymes, usually metalloproteases. Several studies have investigated the haemostatic effects of Viperidae snake venoms and their isolated protein components [10, 16, 37, 40, 41]. Proteolytic enzymes and myotoxins are the central components in the venom of members of the Viperidae family, among which snake venom metalloproteases (SVMPs) induce symptoms such as haemorrhages [36, 45]. These proteases interact with different targets to regulate haemostasis or with important tissues associated with vital physiological functions in prey and predators, causing the most palpable effect: haemorrhages [3]. The different actions of these proteases involve different targets, such as the activation of coagulation factors [43], fibrinogen [14], and the endothelial extracellular matrix of capillary vessels [21].

The taxonomic classification of species in the *Porthidium* genus has been controversial over the past several years. Species of this genus were previously included in the literature as members of the *Bothrops* genus [29] however, based on taxonomic criteria and molecular studies, phylogenetic relationships have been established among Viperidae family members in the *Porthidium*, *Atropoides* and *Cerrophidion* genera in the so-called “Central American Lineage», which is widely distributed in Central America [2, 6, 23]. In this lineage, the *Porthidium* genus is the only genus found in Venezuela [4, 11, 44]; this lineage forms a paraphyletic group of «South American Lineage» genera and comprises *Bothrocophias, Bothrops* and *Bothriopsis* [4, 11, 31].

The main objective of this work was the purification and the biological-biochemical characterisation of a haemorrhagic component found in the venom of the snake *Porthidium lansbergii hutmanni* (*P.l.h*).

MATERIALS AND METHODS

Reagents

The next materials were used for electrophoresis: reagents (Bio-Rad, USA) and immobilized pH gradient (IPG) strips, pH 3-10, 11 centimetres (cm) (Bio-Rad, USA). The following materials were used for haemostasis: human fibrinogen (Sigma, MO, USA) and bovine thrombin (Sigma, MO, USA). These materials were used for immunoblotting: equine peroxidase-coupled-equine IgG antibody (Santa Cruz Biotechnology, CA, USA); nitrocellulose membrane (Bio-Rad, USA); and SuperSignal West Pico® chemiluminescence development kit (ThermoScientific, USA). These materials were used for MALDI-TOF/TOF: α-cyano-4-hydroxycinnamic acid matrix (α-CHCA) (Sigma, MO, USA); ACN; trifluoroacetic acid (TFA); and diethyl ether (Sigma, MO, USA). The next materials were used for LC-MS/MS: OFFGEL RoomTemp HighRes® Kit (Agilent Technologies, USA); IPG strips, pH 3-10, 24 cm strips (GE Healthcare, USA); swine trypsin (PROMEGA); electro spray calibrant solution 63606; and Calibration Tune Mix ESI (Sigma-Fluka, USA). Working solutions were composed of reagents of high purity (≥98%, Merck and Riedel de Haen, Germany). Polyvalent antiophidic serum (PAOS) was obtained from Biotecfar C.A., Caracas, Venezuela.

Software

Prism® (GraphPad, Software) [51] was used for statistical analyses. For one dimension gels analysis and two-dimensional gels electrophoresis analysis QuantityOne® (Bio-Rad, USA)(54) and PDQuest® (Bio-Rad) [48], respectively, were used. For the MALDI/TOF experiment, the Compass 1.2 SR1 for Flex Analysis (BrukerDaltonics)[30} software was employed.

The liquid chromatography (LC)-MS/MS analysis was done with the Compass 1.2 SR1 program for Microtof/Maxis® (Bruker-Daltonics).

Experimental animals

The animals were purchased from the National Institute of Hygiene “Rafael Rangel” (Caracas, Venezuela) animal facility. The mice were kept in cages at room temperature with 12 hours (h) of natural light and *ad libitum* water and food until experimentation. Male mice (*Mus musculus*) of the National Institute of Health (NIH) strain weighing 20 to 22 grams (g) and 25 to 27 g were used to determine lethality and haemorrhagic activity, respectively.

Venom

The pooled *P.l.h* venom was obtained by manually milking 11 adult specimens of both sexes that were captured at 3 metres above sea level (m.a.s.l) in the flat regions of Margarita Island, Nueva Esparta State (Venezuela),The animals were captured in Macanao peninsula, geographically located at Longitude: “O64°16’59.99” and Latitude: “N11°1’0.01”). The area these specimens originate from has a climate favourable to xerophytes; the climate is influenced by northeast trade winds (“vientos aliseos”), the tropical floor as an average annual temperature of 28°C and an annual rainfall less than 800 millimetres (mm), and the vegetation is very similar to the Venezuelan coastal inland vegetation. The majority of this area comprises flat terrain, and common vegetation is the arborescent cacti group known as “Cardonal”, which is mainly characterised by columnar cacti (“cardones”) and spiny Mimosaceae with a squat appearance (“cujíes”). The prevailing vegetation near

the seashore is these “Cardonales”; however, spine bushes or “Espinares” (“cujíes”) are common inland [38].

On the other hand, the *Bothrops colombiensis* venom originated from the pooled venom of 12 adult specimens of both sexes, which were captured in different Venezuelan regions. All snakes were maintained in captivity in the Serpentarium of the Pharmacy School Faculty, Universidad Central de Venezuela (Caracas, Venezuela). Once obtained, the venom was crystallized under vacuum in a desiccator (Pyrex ®, 2.4L Small Knob Top Desiccator Corning, USA) containing $CaCl_2$ as a desiccant and maintained at 4°C (Frigidaire FGVU21F8QF Vertical Freezer, USA) until use.

Fractionation of *P.I.h* crude venom by gel filtration

The fractionation of the *P.I.h* crude venom was initiated with a Sephadex® G-100 molecular sieve chromatography column (90 x 2.5 centimetres (cm) following the method by Grillo and Scannone [18]. Venom samples were dissolved in 5 millilitres (mL) of mobile phase, composed of 0.2 Molar (M) ammonium acetate buffer at pH 6.8 (four runs were performed, for a total of 1000 milligrams (mg of venom). Protein elution was achieved by mobile phase at a flow rate of 7 mL/h. The eluates were monitored at 280 nanometres (nm). Fractions were lyophilised, weighed, and stored at -20°C (Frigidaire FGVU21F8QF Vertical Freezer, USA) until used to evaluate haemorrhagic action. To determine which of the fractions (FI to FIV) obtained from *P.I.h* venom by molecular exclusion had the highest haemorrhagic activity, a single dose of 1 microgram (µg) in 0.1 mL of the FI and FII fractions diluted in 0.85% saline solution was inoculated in experimental animals, and four animals were used per group. The haemorrhagic area was established as described for the determination of the Minimum Haemorrhagic Dose (MHD). FI had the highest activity and was selected for further purification with anionic exchange chromatography.

Anion exchange chromatography

The fractionation was carried out in an automated work station (Bio-rad, USA) by means of an anion exchange column [1] in several stages. Briefly, FI was dissolved in a 20 millimolar (mM) acetic acid/sodium acetate buffer at pH 5.4 (“A” solution). Then, a gradient between solution “A” and an elution buffer solution “B” (20 mM acetic acid/sodium acetate, pH 3.0) was established, with the percentage of the “B” solution increasing by 10% at each step. Finally, the proteins that failed to be eluted under these conditions were subjected to a linear gradient of NaCl at a final concentration of 2 M.

Throughout this process, the flow rate of the mobile phase was 1 mL/minute (min), and protein detection was carried out at 280 nm. A total of 20 runs (100 mg) were performed to obtain a batch of each fraction. Each individual batch was concentrated and desalted by centrifugation at 2,500 x G), using concentrator tubes, with a 3 kilodaltons (kDa) cut-off, until 90% of the volume was reduced. Then, the samples were resuspended in deionised water. This process was repeated three times for each fraction until an aqueous solution with a neutral pH was obtained at an appropriate protein concentration. The fractions obtained at the end of this process were stored at -20 °C (Frigidaire FGVU21F8QF Vertical Freezer, USA) until use.

Selection of the anion exchange fraction of interest

A single dose of 1 µg of protein from each ion exchange fraction was intradermically injected into experimental animals, and four animals were used per group. The fraction with the highest haemorrhagic activity, protein content, and purity as evidenced by one-dimensional electrophoresis was selected for toxicological characterization and proteomic identification.

Protein determination

The protein content in the fractions obtained by anion exchange chromatography was determined [32] using bovine serum albumin as a standard for the calibration curve.

High-performance liquid chromatography of hutmannin-1

The purified fraction (100 µg) was dissolved in 200 µL of 1% TFA in deionised water and subjected to a reverse phase C-18 column on an High Performance Liquid Chromatography (HPLC) (Waters Alliance,) instrument.

A linear gradient from 0 to 100% acetonitrile (ACN) in 0.1% TFA was established over one h at a flow rate of 1 mL/min. The eluates were detected at 280 nm. The appearance of a single, acute and symmetric peak was considered the purity criterion of the component named hut-1.

Identification of hutmannin-1 with MALDI-TOF/TOF mass spectrometry

The identification of hut-1 was carried out at the Toxicology Laboratory, Department of Physiology and Pharmacodynamics, Oswaldo Cruz Institute, Rio de Janeiro, Brazil. For this method, band fragments from SDS-PAGE were treated with 65 mM DL-Dithiothreitol (DTT) (Sigma-Aldrich, USA) for 30 min at 56°C to reduce the protein disulphide bonds, and then the samples were subjected to alkylation with 100 µL of 200 mM iodoacetamide for 30 min. Later, the gel fragments were decolourised with 50% ACN in 25 mM ammonium bicarbonate at pH 8.0, dehydrated with 200 µL of ACN and trypsinised with 15 µL of a (20 ng/µL) trypsin solution, prepared in 40 mM ammonium bicarbonate. The obtained peptides were analysed by MALDI-TOF/TOF mass spectrometry [14, 39]. The mass spectrum was obtained, and *de novo* sequencing of the analysed peptides was carried out. The obtained sequence was compared with the protein sequences deposited in the NCBI (National Center for Biotechnology Information, USA) with the BLAST program.

Assessment of FI and hutmannin-1 haemorrhagic potency

at each purification step

The performance of FI and hut-1 was defined as the per-cent increase in haemorrhagic potency at each purification step.

SDS-PAGE analysis of venom

SDS-PAGE (12% gel) was carried out [28]. Briefly, samples were dissolved at a concentration of 5 µg/µL in a protease inhibitor cocktail composed of 4-(2-aminoethyl)-benzene-sulphonyl fluoride (AEBSF), E-64, bestatin, leupeptin, aprotinin and disodium

EthylendiamineTetraacetic acid (EDTA), and then the samples were diluted to the optimum concentration for visualisation in 0.5 M Tris-HCl buffer at pH 6.8, 10% SDS, 1% glycerol and 0.02% bromophenol blue. For hut-1, a sample under reduced conditions was also prepared. Once loaded in their respective gel wells, the samples were run at 100 V for approximately 120 min. Afterward, the appropriate gels were selected for Blue Silver staining [5], which has a sensitivity of 1 ng per band. Next, the gels were washed with deionised water to remove excess dye and digitised. Each experiment was performed in triplicate.

Hutmannin-1 lethality

Venom lethality (deaths and signs of toxicity) was determined in mice intravenously injected with 50 µg or 25 µg of hut-1 samples, corresponding to doses of 2.5 mg/kg and 1.25 mg/kg, respectively. These doses were selected on basis of the lethality of the crude venom and FI. The animals were autopsied, and the macroscopic observations of the haemorrhages were performed.

Determination of the minimum haemorrhagic dose (MHD)

To determine the MHD of the *P.l.h* hut-1, a modified method [27] was used. Serial doses of hut-1 in the range of 0.0088 µg to 0.044 µg were intradermally injected into the depilated backs of mice (*Mus musculus*). The mice were sacrificed, and the skin was removed after 2 h. The diameter of the haemorrhage on the skin was measured, and the MHD was defined as the amount of venom protein required to induce a 10 mm haemorrhage.

With the experimental data, a dose response graph was constructed. Linear regression analysis was performed to estimate the MHD from the equation line with Prism® (GraphPad). This procedure was repeated in triplicate, and the mean and standard deviation of the MHD were calculated.

Effect of temperature on hutmannin-1 activity

Hutmanin-1 was prepared in 0.1 mL of 0.85% saline solution such that the amount of protein corresponded to 10x MHD. From this solution, 2 mL aliquots were incubated for 30 min at different temperatures: 40, 50, 60, 70, 80, and 90°C. After the incubation period, 0.1 mL of each sample was injected into groups of four mice. The diameter of the produced haemorrhagic area was calculated as indicated for the determination of the MHD. The per-cent haemorrhagic activity was calculated relative to the activity of the control, which was a sample incubated for 30 min at 30°C.

Determination of procoagulant activity on human plasma

The ability of *P.l.h* crude venom and hut-1 to induce blood coagulation was determined through the physical observation of clot formation [47] Briefly, different venom or fraction dilutions were prepared in a coagulation solution composed of 0.02 M phosphate-saline buffered solution (PBS) at pH 7.4. Aliquots of 50 µL of crude venom and hut-1 dilutions were added to 200 µL of citrated human plasma from healthy laboratory donors. In the case of crude venom, concentrations ranging from 0.1 µg to 100 µg per 50 µL were used. For hut-1, the concentration ranged from 5 to 40 µg. The coagulation time was recorded, and the samples that induced plasma coagulation in less than 30 min were considered procoagulants.

Four replicates were carried out for each dilution. Additionally, four tubes of the coagulation control solution without *P.l.h* crude venom were prepared. A sample 5 µg of *B. colombiensis* venom and 200 µL of plasma as a positive control was also carried out, in which the coagulation time should not exceed 60 seconds (sec).

Anticoagulant activity determination

In addition to the previous experiment, whether the *P.l.h* crude venom or hut-1 inhibited or promoted plasma coagulation when recalcified was determined [13]. The procedure consisted of making dilutions of venom or hut-1 preparation in coagulation solution to contain the required dose in 50 µL of solution. With crude venom, doses ranging from 0.1 µg to 100 µg were used. For hut-1 doses from 5 µg to 40 µg, 50 µL of each dilution was added to 200 µL of citrated plasma and incubated at 37°C for 10 min. During this interval, it was observed if plasma coagulation occurred. If not, 100 µL of 1 M $CaCl_2$ was supplemented to the tube and again incubated, recording the coagulation time for another 30 min. Four replicates were made for each trial. The experimental control consisted of 50 µL of 0.85% saline solution and incubated with plasma in the absence of venom or hut-1.

Determination of defibrinating activity

The ability of venom or fractions to degrade fibrinogen *in vivo* was assessed [13]. Briefly, groups of five (20-22 g) mice were intravenously injected with different dilutions of *P.l.h* crude venom (7.5 µg to 120 µg) or a single dose of 25 µg of hut-1, prepared in 0.2 mL 0.85% saline solution. One hour after the injection, blood was drawn from the axillary plexus of each experimental animal under anaesthesia. Samples were stored in glass tubes for two h at room temperature, and clot formation was observed. The minimal defibrinating dose (MDD) was defined as the minimum amount of venom that induced incoagulability in all inoculated mice. In addition, a control group was inoculated with 0.85% saline solution and developed a firm clot one h after collection.

Determination of the fibrinogenolytic activity of *P.l.h* crude venom and hutmannin-1

To evaluate the proteolytic activity of *P.l.h* crude venom and hut-1 on the α, β, and γ chains of fibrinogen, we proceeded according to a modified protocol [34]. In brief, a stock solution of purified human fibrinogen was prepared at a concentration of 2 mg/mL in a 0.1 M Tris-HCl buffer solution at pH 7.4. Different concentrations of *P.l.h* crude venom (0.03 µg to 2 µg) and hut-1 (0.5 µg to 32 µg) were incubated at 37°C for 30 min with pre-prepared aliquots of fibrinogen solution (50 µL/100 µg). After incubation, each sample was diluted 1:1 with reducing solution containing 0.5 M Tris at pH 6.8, 10% SDS, 1% glycerol, 0.02% bromophenol blue, and 3% 2-β-mercaptoethanol. Then, the samples were placed in a water bath (Whip Mix, WPM-05350, USA) at 100°C for 5 min. An aliquot of 15 µL of each sample was electrophoresed as indicated. The degradation of different fibrinogen chains was observed. A fibrinogen sample was run under the same conditions in the absence of *P.l.h* crude venom or hut-1.

Determination of fibrinogenolytic activity as a function of time

After establishing the lowest amount of crude venom (0.25 µg) and hut-1 (1 µg) capable of completely degrading the α chain of human fibrinogen under the conditions described above, the appropriate concentrations of venom and hut-1 were incubated with 100 µg of fibrinogen at 37°C for the following incubation periods: 30 seconds, 1 min, 5 min, 15 min, 30 min, 60 min, 3 h and 24 h. Subsequently, to determine the fibrinogenolytic activity, these samples were electrophoresed (SDS-PAGE) as indicated and compared to the electrophoretic pattern of a control sample consisting of the corresponding dose of crude venom or fraction, incubated with 100 µg of fibrinogen and immediately subjected to the reducing action of the reducing solution (time 0).

Effect of protease inhibitors on fibrinogenolytic activity

Constant amounts of *P.l.h* crude venom (1 µg) and hut-1 (2 µg) were incubated at 37°C for 30 min with 100 µg of human fibrinogen in a 0.02 M Tris-HCl buffer solution at pH 7.5. To evaluate the fibrinogenolytic serine protease activity, 2 mM benzamidine was added to the incubation mixture, whereas to evaluate the metalloprotease activity, EDTA was added to the samples. As controls, samples without protease inhibitors were used. After the incubation period, the samples were evaluated by SDS-PAGE as indicated to determine fibrinogenolytic activity.

Effect of the pH on fibrinogenolytic activity

A constant dose of hut-1 (50 µL/3 µg) was prepared in the following buffer solutions at different pH values: citric acid/0.1 MNa2HPO4 (pH 3, pH 4, pH 5 and pH 6) and 0.1 M Tris-HCl (pH 7, pH 8, pH 9 and pH 10). The mixtures were incubated at 37°C for 30 min with (50 µL/100 µg) human fibrinogen, and then SDS-PAGE was performed as indicated to determine fibrinogenolytic activity.

Determination of fibrinolytic activity

The ability of *P.l.h* crude venom and hut-1 to degrade fibrin was determined [34]. Briefly, 1.5 mL of 0.1% fibrinogen solution in imidazole-buffered 0.85% saline solution at pH 7.4 was added to Petri dishes (3 cm). Then, 75 µL of 10 U/mL bovine thrombin containing 0.025 M $CaCl_2$ was added to form a uniform fibrin layer. Afterward, 10 µL (1 µg/µL) of crude venom or 10 µL of (1 µg/µL) hut-1 in 0.85% saline solution was placed in the centre of the fibrin layer and incubated for 24 h at 37°C. After the incubation period, the diameter of the lysis area on the fibrin surface was determined. Fibrinolytic activity was expressed as the diameter (mm^2) of the lysis area per microgram of venom or fraction.

Determination of proteolytic activity on gelatine

The modified methodology proposed by Terra *et al.* [46] was followed for this step. Discontinuous 12.5% polyacrylamide gels copolymerised with 1% gelatine were run. Then, 3 mL of each sample of *P.l.h* crude venom and hut-1 at a concentration of 2 µg/µL in 0.5 M Tris buffer at pH 6.8, 10% SDS, 1% glycerol and 0.02% bromophenol blue, was added to the gel. In addition, as a positive control, 1 µL (2 µg/µL) of *B. colombiensis* venom was added to the gel. After electrophoresis, the gel was equilibrated in a 2.5% Triton X-100 solution, stirring for 1 h at room temperature, washed with double distilled water (two washes of 10 min each) and incubated at 37°C for 18 h in a buffer solution of 20 mM Tris-HCl at pH 7.4, 150 mM NaCl, and 5 mM CaCl2. The proteolytic activity on gelatine was evidenced by the zones of degradation in the gel, which were observed as translucent areas after Coomassie R-250 blue staining. [5].

Determination of the effects of *P.l.h* crude venom and hutmannin-1 on platelet aggregation

The effects of venom and hut-1 on platelet aggregation were assessed by the turbidimetry method [7]. Briefly, blood was obtained from healthy laboratory donors and centrifuged at 190 x G and 20°C for 15 min to obtain platelet-rich plasma (PRP). After counting platelets, an aliquot was subjected to a second centrifugation at 1700 x G for 15 min to obtain the platelet-poor plasma (PPP). The plasma used during the trials consisted of the PRP at a concentration of $1x10^5$ platelets/mL adjusted with the PPP.

Each measurement was obtained in an aggregometer (Crono-Log®560, USA), and aliquot suspensions of 500 µL were placed under agitation at 37°C in a silicon cuvette during the determination. A total of 10 µL of different dilutions of crude venom (0.6 µg to 16 µg), prepared in normal saline solution, were added to each sample. For hut-1, an amount corresponding to five times the IC_{50} of crude venom value was used. After 4 min, the aggregation agonists ADP (10 µM), ristocetin (1.25 mg/mL), collagen (8 µg/

mL) and thrombin (1 U/mL) were added. The aggregation curve was recorded over 8 min for all assays. As a reaction control, the agonists were placed on the platelet suspension without venom and instead adding 10 µL of 0.85% saline solution. In the case of crude venom, a dose response curve was prepared with the obtained results, and IC_{50} of each agonist was determined. The IC_{50} was defined as the amount of venom capable of reducing platelet aggregation by 50% with respect to the control.

Neutralisation and antigenic recognition assays of *P.l.h* crude venom and hutmannin-1 by the polyvalent anti-**ophidic serum**

Neutralisation of haemorrhagic activity

The antivenom used in the neutralisation experiments was PAOS, produced by the Biotechnology Centre of the Faculty of Pharmacy of the Universidad Central de Venezuela (Biotecfar C. A), lot L-162. PAOS consists of $F(ab)_2$ fragments of hyperimmune immunoglobulins obtained from horses. *(Equus ferus caballus)* The species used for the immunisation were *Crotalus durissus cumanensis, Crotalus vegrandis, Crotalus pifanorum, Crotalus ruruima, Bothrops atrox, B. colombiensis, Bothrops venezuelensis,* and *P.l.h*.

The capacity of PAOS neutralising the haemorrhages induced by *P.l.h* crude venom, FI and hut-1 was determined. Briefly, crude venom, F1 or hut-1 was combined with different antivenom dilutions, using as a reference value of the titre declared by the PAOS manufacturer for *Bothrops* genus (1 mL of PAOS must neutralise the activity of 2 mg of *Bothrops* venom).

The neutralising test was prepared to obtain 10 MHD in 0.1 mL of the mixture and different venom/antivenom proportions of crude venom, F1 or hut-1. The mixtures were incubated for 30 min at 37°C and centrifuged at 2,500 x G for 10 min to eliminate the antigen-antibody complexes that formed.

The experimental animals were assigned to groups of five mice each. Each mouse in the groups was injected with 0.1 mL of the appropriate venom/antivenom mixture.

Additionally, there was a venom control group challenged with the crude venom or fraction and a serum control group that received the highest PAOS dose used in the experiments. Two h after the injection, the haemorrhagic lesion as described for the MHD was evaluated. The per-cent reduction in the haemorrhagic lesion diameter induced by each dose was calculated with respect to the control. The ED_{50} was defined as the amount of PAOS capable of reducing the diameter of the haemorrhagic lesion by 50%.

Immunoblotting assays

The PAOS reactivity against the epitopes present in the *P.l.h* crude venom and hut-1 were evaluated using western immunoblotting. In this determination, the selected gel was incubated for 10 min in transfer solution (50 mM Tris-HCl at pH 8.0, containing 380 mM glycine, 0.1% SDS and 20% methanol). Then, the gel was placed in a transfer chamber, allowing the proteins to pass from the polyacrylamide matrix to a nitrocellulose membrane. This process was carried out at 180 milliampers (mA) for 2 h.

After the transfer, the nitrocellulose membrane was blocked for 2 h at room temperature with a 0.2 M PBS solution at pH 7.0, with 5% (w/v) skimmed milk and 0.1% (w/v) Tween 20. Later, three washes were performed for 5 min, each with a solution of 0.05% (w/v) Tween 20 and 0.2 M PBS at pH 7.0. The membrane was incubated again at room temperature for another 90 min, with PAOS diluted to 1:3000 in blocking solution. After the incubation period, the membrane was washed three times with the washing solution for five min each wash. Immediately after the washes, the secondary antibody anti-equine IgG (coupled to horseradish peroxidase) diluted 1:7000 in blocking solution was added. Then, the membrane was incubated at room temperature for another 90 min and washed, as indicated. The electrophoretic bands recognised by PAOS were visualised using a chemiluminescence development kit, and the image was analysed.

Effect of protease inhibitors on the haemorrhagic activity of hutmannin-1

The effect of protease inhibitors on the haemorrhagic activity of hut-1 was tested using EDTA, a metalloprotease activity inhibitor, and benzamidine, a trypsin or trypsin-like inhibitor. In each case, the proteases were preincubated with the corresponding inhibitor at 37°C for 30 min.

Six experimental groups, consisting of four mice each, were used. Each mouse was inoculated with dose of hut-1 that corresponded to 5 times the MHD. The first group received hut-1 preincubated with 2 mM EDTA, the second group was inoculated with hut-1 preincubated with benzamidine, and the third group received a dose of hut-1 preincubated in 0.85% saline solution, representing the haemorrhage control group. The remaining groups were inoculated with the following vehicles: 0.85% saline NaCl, 2mM EDTA and 2 mM benzamidine. Each animal was injected and the haemorrhagic area was determined as indicated to determine MHD. The arithmetic mean was calculated for the results of each group. The per-cent reduction induced by the protease inhibitors was calculated by considering the diameter of the haemorrhagic area for the crude venom control group as 100%.

Statistical analysis

The MHD and neutralisation were analysed by linear regression. The data were expressed as the mean ± standard deviation. To determine differences between experimental groups (three replicates of each condition), one-way ANOVA with Dunnett's post hoc test was used to compare the experimental conditions to the control conditions. Results with an error probability <0.01 were

considered significant. Analysis was completed using SPSS version 2.0 [35]

RESULTS AND DISCUSSION

The majority of snake venoms exert their actions on almost all tissues, and their pharmacological activities are determined by several biologically active fractions [21]. The most significant components of these bioactive fractions are SVMPs, the major components of the venom produced by the *Porthidium* genus [23, 29]. SVMPs are enzymes considered to have the highest haemorrhagic potential among the components of Viperidae snake venoms. These proteins are capable of degrading extracellular matrix proteins such as laminin, nidogen, fibronectin, type IV collagen (constituents of vessel walls) and proteoglycans in the endothelial basement membrane, which promotes the diffusion of venom through the membranes and weakens the capillary structure. Together with the hydrostatic pressure generated inside the blood vessel, SVMPs can produce blood extravasation [9]. Other toxic activities attributed to these enzymes include fibrinogenolytic activity, prothrombin and Factor X activation, apoptosis induction, platelet aggregation inhibition, proinflammatory activity, and inactivation of serine protease blood inhibitors [26, 33]. Most Viperidae snake venoms alter blood coagulation, but there are a few venoms, such as those produced by *Bothriechis lateralis* and *Porthidium nasutum*, that do not alter blood coagulation; however, these venoms do induce haemorrhages due to other protease activities [19] According to the current analysis of *P.l.h* crude venom, only high concentrations of F1 showed procoagulant activity, indicating that the proportion of proteins with procoagulant activity is low in this venom.

Other researchers [25, 37] have reported that mammalian experimental models with disrupted platelet aggregation did not present abnormalities in coagulation tests, despite showing signs of systemic haemorrhaging.

In the size exclusion chromatography, four protein peaks from the *P.l.h* venom were obtained (data not shown). Two predominant fractions were isolated (FI and FII) and used for further purification. These fractions presented the largest areas in the chromatogram. FI (1 µg) produced the largest haemorrhagic area (22.29 ± 2.79 mm) on mouse skin and was selected for the next purification stage using anion exchange chromatography. FII at this dose did not produce any haemorrhagic lesions, and it was discarded.

After passing FI through an anion exchange chromatographic column, seven well-defined peaks were obtained [FIG.1]. A total of 20 runs were carried out, and the fractions (named according to the percentage of buffer "B" composing the mobile phase) were grouped and homogenised as follows: first, a fraction called F0% (tubes 5 and 6), whose protein content did not interact with the negatively charged resin. Second, a series of fractions were bound to the resin with different affinities, according to their isoelectric

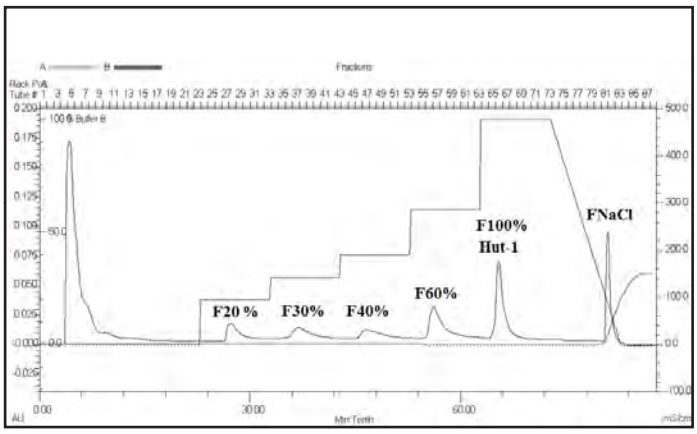

FIGURE 1. PURIFICATION OF HUT-1 BY ANION EXCHANGE CHROMATOGRAPHY. Fraction I obtained from the crude venom of *P. lansbergii hutmanni* was applied to a Q1 column (BioRad, USA). Elution was performed by establishing a pH step gradient with buffer pH 3 in five steps (20, 30, 40, 60 and 100%) during first 73 min of the run (showed on X axis), following with a NaCl 2M gradient, evidenced by an increasing of conductivity value expressed in mS/cm. The fraction with the highest haemorrhagic activity was called hut-1 and was selected to check its homogeneity by means of HPLC.

Mice were intradermally injected with 1 µg of the protein from the anion exchange fractions, and the haemorrhagic lesions that resulted from these injections are shown in TABLE I. The fractions F40%, F60%, F100% and FNaCl had a strong haemorrhagic action. The fraction F100% was selected for further biochemical and toxicological characterisation because it has the highest haemorrhagic activity.

TABLE I
HAEMORRHAGIC LESIONS PRODUCED BY ANION EXCHANGE FRACTIONS

Anion exchange fraction	Haemorrhagic lesion (10 mm diameter) (µg protein/fraction)
F0%	NH
F20%	NH
F30%	NH
F40%	20.7 ± 0.5µg
F60%	20.9 ± 0.6µg
F100%	22.5 ± 0.3µg
FNaCl	17.8 ± 0.8µg

NH: No haemorrhages were observed.

It was possible to purify a 62 kDa protein called hut-1 using a combination of size exclusion chromatography and anion exchange chromatography. Hut-1 showed a single protein peak that eluted

during the acetonitrile (ACN) gradient under high-performance liquid chromatography (HPLC). As shown in FIG.2, this peak was symmetric, and no major peaks corresponding to protein sample contaminants were observed. This protein was composed of a single polypeptide chain, as evidenced by treatment with the reducing agent B-mercaptoethanol. Hut-1 was identified as a member of the SVMP family, with homology to class P-III of the zinc-dependent metalloprotease domain family based on the molecular mass determined by tandem mass spectrometry (MS/MS). The toxic action of hut-1 demonstrated that, similar to other SVMPs [45], its main toxicological targets were haemostasis components.

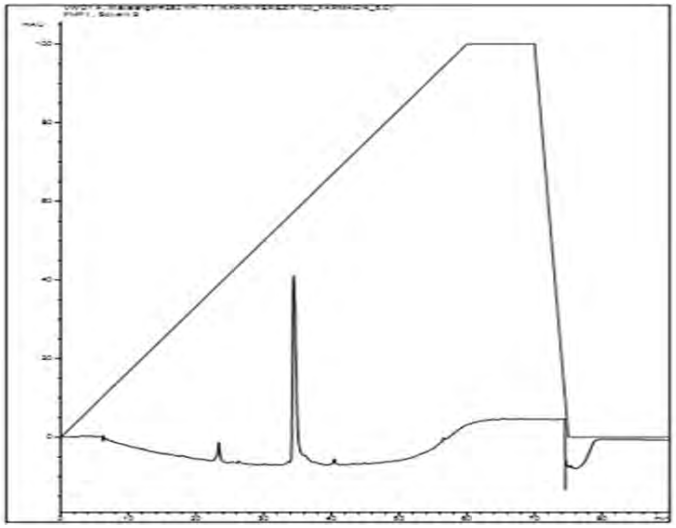

FIGURE 2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) OF HUT-1. Hutmannin-1 (100 µg) was dissolved in 0.1% trifluoroacetic acid (TFA) and run in a C-18 reverse phase column (HPLC) with a linear gradient from 0 to 100% acetonitrile (ACN), in one h at a flow rate of 1mL/min.

Exploring the lethality of *P.l.h* FI and hutmannin-1 was obtained that the LD_{50} of FI (1.45 ± 0.16 mg/kg) was lower than that of the crude venom of *P.l.h* (2.51 ± 0.16 mg/kg), showing that this fraction possessed the majority of the toxic components in the venom. Hut-1 showed high toxicity. It was not feasible to determine the LD_{50} of hut-1 because it was not possible to obtain enough sample for this test; however, when doses as low as 2.63 mg/kg and 1.32 mg/kg were tested, all the animals died within 24 h after the injection, which indicated that the hut-1 LD_{50} was below 1.32 mg/kg. Before the animals injected with both doses of hut-1 died, they all presented motor incoordination, flaccid paralysis in the anterior and posterior limbs, cyanosis, respiratory insufficiency, tachycardia and haematemesis. Autopsies of mice revealed atrial thrombosis and massive pulmonary and hepatic haemorrhages, indicating the high toxicity of hut-1 (data not shown).

The acute toxicity of hut-1 was found to be more potent than those of FI and crude venom. This toxicity is notable when comparing hut-1 with a PI class metalloprotease (Porthidin-1), previously isolated from *P.l.h* venom [15], which was found to be non-lethal in experimental mice intravenously injected with a dose of 6 mg/kg.

The results obtained in each purification step are shown in TABLE II. Here, the performance and efficiency of the purification process of hutmannin-1 (hut-1) were determined, and the minimum haemorrhagic dose (MHD, µg) was obtained at each purification step.

The MHD of hut-1 was 83 times higher than that of crude venom, showing that hut-1 is one of the most powerful haemorrhagic toxins described to date; this conclusion becomes evident when hut-1 is compared with similar toxins from the venoms of various Viperidae snakes [49]. Moreover, the autopsies of mice treated with hut-1 showed extensive systemic haemorrhages. The macroscopic study of the lung tissue of treated mice revealed profuse haemorrhages in the lung, and these lesions rapidly appeared 15 min after injection. Observations of the structural changes that occur following exposure to *P.l.h* crude venom [49] revealed the appearance of erythrocytes and glomerular kidney oedema, as well as the detachment from the basement membrane and plasma membrane rupture of endothelial cell. This result could demonstrate the possible systemic haemorrhagic activity of hut-1, the haemorrhagic fraction from this venom. Previous studies that investigated other metalloproteases from *Bothrops* snakes, such as jararagin, have shown that similar pulmonary haemorrhages occur in mice [8, 36]. The MHD value is shown in TABLE II. At nanogram (0.0088 to 0.044 µg) doses, hut-1 presented a high haemorrhagic capacity, resulting in bleeding skin lesions in the experimental animals (data not shown).

TABLE II

PERFORMANCE AND EFFICIENCY OF THE PURIFICATION PROCESS OF HUT-1. THE MHD ACTIVITY (µG) RESULTS OBTAINED AT EACH PURIFICATION STEP

Sample	Amount obtained	MHD activity (µg)	Purification factor
Crude venom	1000 mg	1.475	1
Fraction I	697.2 mg	0.102	14.5
Hutmannin-1	39 mg	0.021	69.9

Hut-1 maintained its haemorrhagic activity when incubated for 30 min at 40°C, but haemorrhagic activity of hut-1 decreased by approximately 20% after incubation at 50°C for the same period of time. After exposure to temperatures equal to or higher than 60°C, hut-1 completely lost its haemorrhagic capacity [FIG. 3A]. This temperature restriction is similar to the temperature restriction of other viperid metalloproteases, such as uracoin-1 [1], and elapid metalloproteases, such as EpyHTI and EcoHTI, which presented a 50% reduction in haemorrhagic activity at temperatures close to 50°C and completely lost activity at 70°C [49].

The haemorrhagic activity of hut-1 was completely abolished by preincubation with 2 mM EDTA, a metal chelator, whereas preincubation with 2 mM benzamidine, a trypsin or trypsin-like inhibitor, did not induce significant differences from untreated hut-1 (n=4, FIG. 3B), demonstrating the dependence of the activity of hut-1 on divalent ions, as previously reported for a large variety of metalloproteases [7,15,16,19, 36, 46].

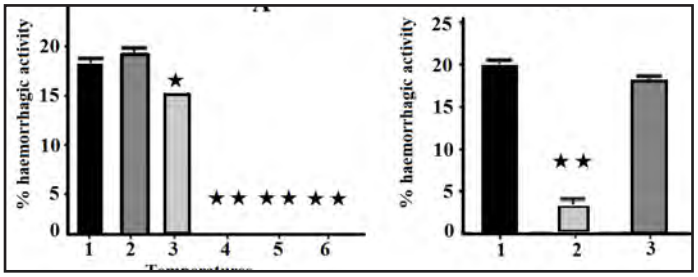

FIGURE 3. EFFECT OF TEMPERATURE AND PROTEASE INHIBITORS ON THE HAEMORRHAGIC ACTIVITY OF HUT-1. (A) 1: Control; 2: 40ºC; 3: 50ºC; 4: 60ºC; 5: 70ºC; 6: 80ºC. The bars represent the standard error. Dunett test one-way ANOVA analysis = * α <0.05 ** α <0.01. **(B)** 1) control; 2) Hut-1 + 2 mM EDTA; 3) Hut-1 + 2mM benzamidine. The bars represent the standard error. Dunett test one-way ANOVA analysis = ** α <0.01

The haemorrhagic potential of high class P-III metalloproteases has been attributed to two main facts: (1) the inability of α2-macroglobulin to inhibit these toxins and (2) the presence of domains with disintegrin-like activity that are rich in cysteine residues and specifically degrade extracellular matrix components, especially type IV collagen [22].

The identification hutmannin-1 by tandem mass spectrometry showed that the fragmentations with matrix assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOF) resulted in two main signals: m/z 3182.95 and 2197.84. The defragmentation of these peptides and their subsequent *de novo* sequencing led to the identification of two peptide sequences, NLLVAVTMAHELGHNL (m/z: 3182.95) and VECETGECC (m/z: 2197.84), which are sequences of zinc-metalloprotease domains found in SVMPs.

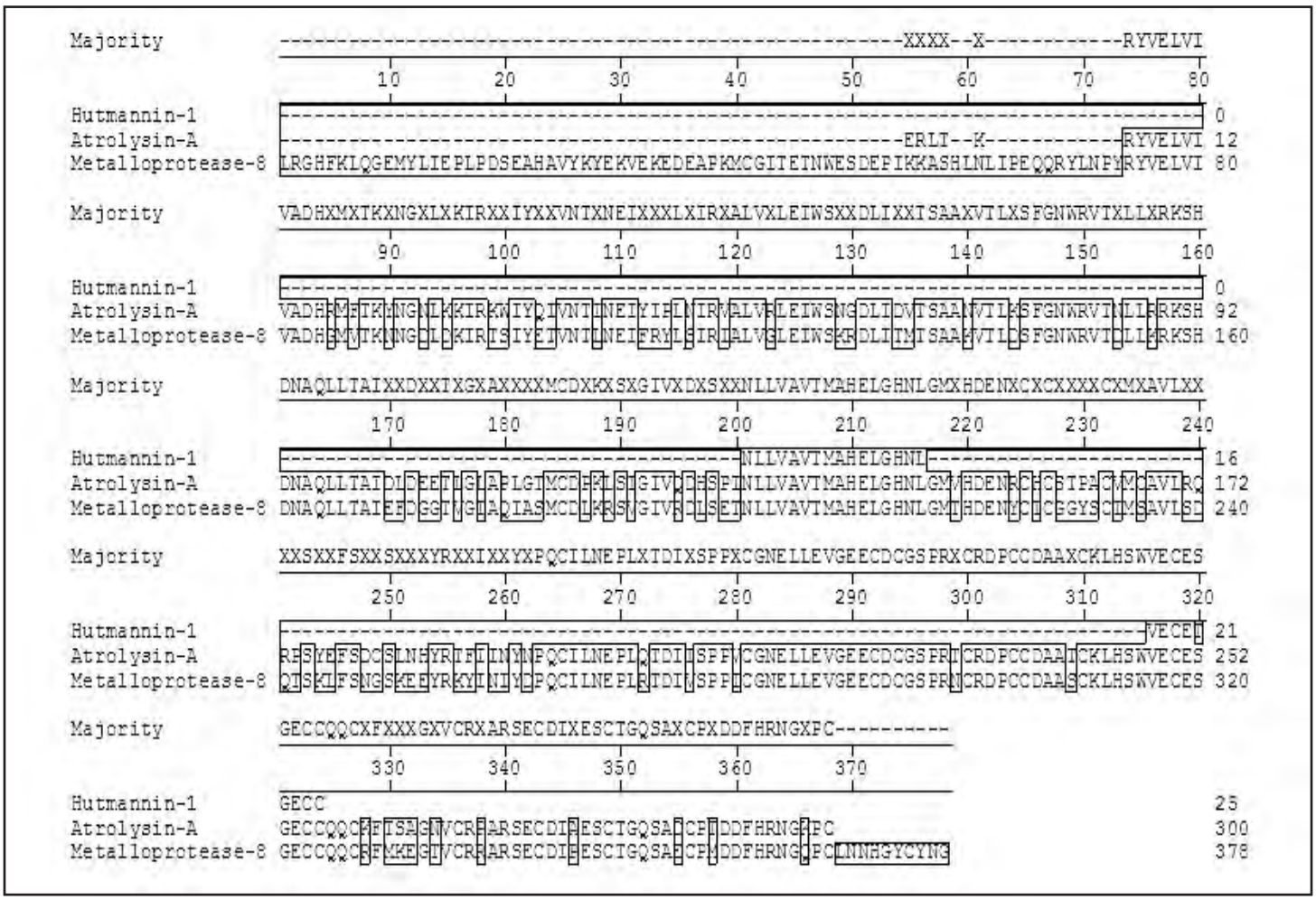

FIGURE 4. NCBI/BLAST search comparison of arrangements *de novo* peptide NLLVAVTMAHELGHNL and VECETGECC sequenced from MS/MS spectrometry, of Hutmannin-1 to partial amino acid sequences of two snake venom metalloproteases, atrolysin-A 17 and metalloprotease-8 [18]. The sequence similarities are shown in the figure

After analysing these sequences with the BLASTp program, hut-1 was identified as an SVMP, showing similarity with adamalisin-1 (*Crotalus adamanteus*), atrolysin-A (*Crotalus atrox*) (E-value 9e-07) [23], metalloprotease-8 (*C. adamanteus*) (E-value 5e-07) [41], and an additional 43 SVMPs [24], all with E-value estimates that are considered significant (≤1e -04). The similarity of the sequences obtained with the sequences of these proteins is shown (FIG. 4).

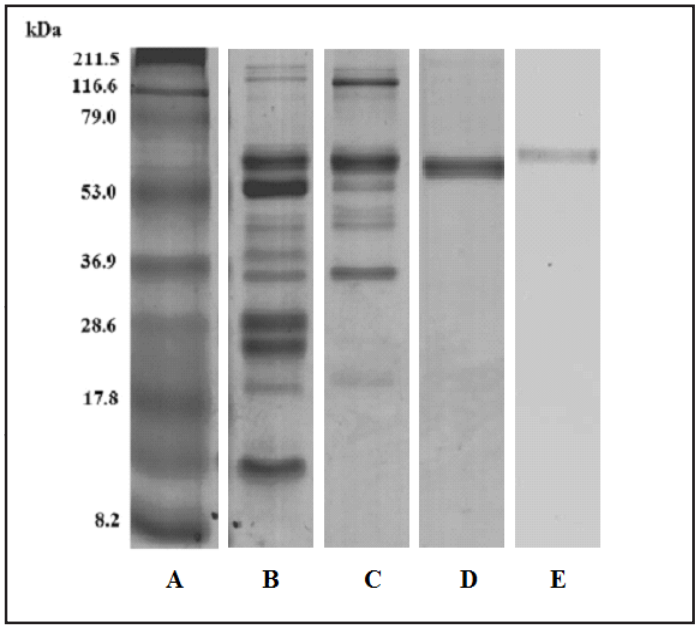

FIGURE 5. *Porthidium l. hutmanni* SDS-PAGE. A) molecular weight markers; B) crude venom; C) FI; D) hutmannin-1 under native conditions; E) hutmannin-1 under reduction conditions. All samples were run with 25µg of protein.

After hut-1 was reacted with the reducing agent 2-β-mercaptoethanol and subsequently underwent electrophoresis, a single band with a slightly higher molecular weight than the unreduced protein (~ 69 kDa) was observed. This result suggested that hut-1 is composed of a single polypeptide chain.

In the procoagulant activity of FI and hutmannin-1 was demonstrated that FI of the *P.l.h* venom dose-dependently induced the coagulation of human plasma, as measured by physical clot formation; all the tested FI doses were markedly more effective than 5 µg of *B. colombiensis* venom, in which the plasma coagulation time was 40 sec (data not shown). Hut-1 did not show plasma procoagulant activity during 30 min of incubation (data not shown). On the other hand, Hut-1 did not show anticoagulant activity on human plasma. Compared to the control, hut-1 did not significantly affect the recalcified plasma coagulation times at the highest doses tested 200 µg (data not shown). The experimental results indicated that mice treated with FI or hut-1 maintained their coagulant capacity 2 h after the intravenous injection of each fraction. This result shows that FI and hut-1 lack defibrinating activity.

In the fibrinogenolytic activity assay, *P.l.hutmanni* crude venom degraded the fibrinogen Aα chain at the concentration of 0.5 µg venom/100 µg fibrinogen (data not shown). Moreover, hut-1 proved to have potent proteolytic action on the fibrinogen α chain at the same hut-1/fibrinogen ratio of 0.5 µg/100 µg. However, it was not easy to determine whether the degradation of the A chain was complete at this ratio; at the 0.5 µg/100 µg ratio, Coomassie blue staining revealed that the hut-1 band was located at the

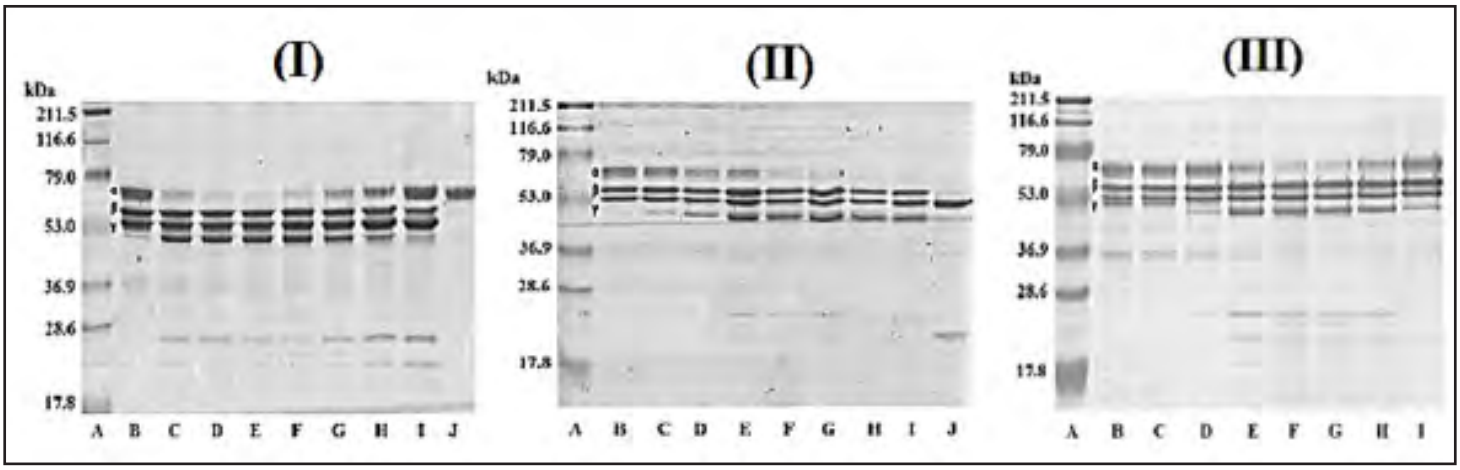

FIGURE 6. (I) FIBRINOGENOLYTIC ACTIVITY OF HUT-1/FIBRINOGEN (µG/µG). A) molecular weights; B) 0 µg/100µg; C) 0.5µg/100µg; D) 1.0µg/100µg; E) 2.0µg/100µg; F) 4.0µg/100µg; G) 8.0µg/100µg; H) 16.0µg/100µg; I) 32.0µg/100µg; J) hut-1 (32µg) in the absence of fibrinogen. **(II) FIBRINOGENOLYTIC HUT-1 EFFECT AS A TIME FUNCTION.** A) molecular weight markers; B) Control (time 0); C) incubation for 30 sec; D) incubation for 1 min; E) incubation for 5 min; F) incubation for 15 min; G) incubation for 30 min; H) incubation for 1 h; I) incubation for 3 h; J) incubation for 24 h. **(III) EFFECT OF PH FOR THE ACTIVITY OF HUT-1 ON FIBRINOGEN AT 37°C INCUBATED FOR 30 MIN.** A) molecular weight markers; B) pH 3; C) pH 4; D) pH 5; E) pH 6; F) pH 7; G) pH8; H) pH 9; I) pH 10

same position as the Aα chain gel due to the similar molecular weights of these proteins. The degradation of the B or y chain was not observed at any of the tested doses [FIG. 6I]. The effect of hut-1 on the fibrinogen α chain was incubation time-dependent. After incubating 1 µg of hut-1 with 100 µg of fibrinogen for different time intervals, the degradation of the fibrinogen α chain was observed. After 30 min of incubation, the fibrinogen Aα chain began to degrade, and it was undetectable after 3 h of incubation. After 24 h, the Bβ chain was completely degraded [FIG. 6II].

Hut-1 lacks the procoagulant activity of other P-III class metalloproteases, such as the Factor V activator RVV-X and the prothrombin activators ecarin and carinactivase-1 [26], and the defibrinating activity associated with the exacerbated activation of some *Porthidium* venom coagulation factors, such as Porthidin-1 [14]. Additionally, the fraction from which hut-1 was isolated FI showed a procoagulant effect at high concentrations, suggesting the presence of other low potency procoagulant toxins. The effect of hut-1 on human fibrinogen was similar to that of crude venom on human fibrinogen, indicating the strong degradation power of hut-1 on the α subunit of fibrinogen. In addition, the β chain was completely degraded after 24 h of incubation. The optimum pH of this activity was between 7 and 9, similar to that of colombienases 1 and 2, as previously reported [17] [FIG.6III].

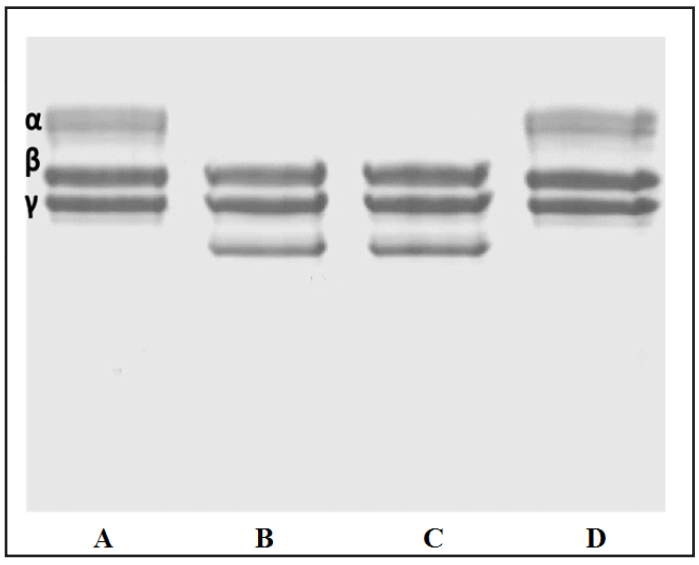

FIGURE 7. EFFECT OF PROTEASE INHIBITORS ON THE FIBRINOGENOLYTIC ACTIVITY OF HUT-1. A) Fibrinogen (Fb) (100µg); B) Fb + hut-1 (100 µg+ 32 µg) ; C) Fb + hut-1 (100 µg+ 32 µg) + 2mM benzamidine ; D) Fb + hut- 1 (100 µg+ 32 µg) + EDTA 2mM.

Alternatively, the degradation of fibrin mesh induced by hut-1 proved to be less effective than that induced by the zymogen. The lysis area obtained in the fibrin plates was 3.5 times

smaller than that obtained with crude venom, wh

ich suggests that toxins other than hut-1 were involved in the fibrinolytic activity of *P.l.h* venom [FIG.8]. Hut-1 showed more activity against the fibrinogen molecule than against the polymerised fibrin mesh.

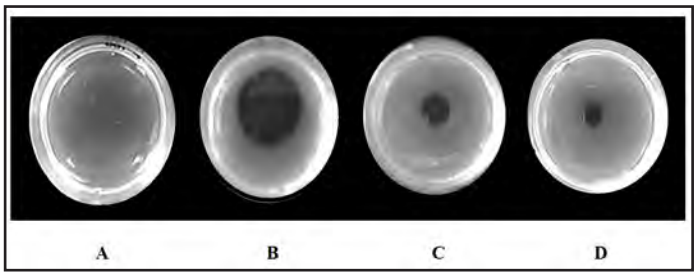

FIGURE 8. FIBRINOLYTIC ACTIVITY OF HUT-1. A) Negative control PBS; B) Positive control *B. colombiensis* crude venom; C) *P.l.hutmanni* crude venom; D) Hut-1.

No proteolytic activity of hut-1 on the hydrolysed collagen that forms gelatine was observed [FIG.9].

Similarly, several authors have correlated gelatinolytic activity with the haemorrhagic action of SVMPs [8,39,41,42]; however, some P-III metalloproteases, such as alsophinase [50] and VLH2 [20], have high fibrinogenolytic and haemorrhagic activity but have not been shown to have gelatinolytic activity [12] [FIG.9]. In the case of hut-1, it was necessary to evaluate this activity by using different forms of colparticularly type IV collagen, as collagen is one of the main toxicological targets for these enzymes. However, when the hut-1 precursor FI was tested, two lysis areas corresponding to ~ 37 and 27 kDa were clearly evident, but no activity was observed in the region corresponding to the molecular weight of hut-1.

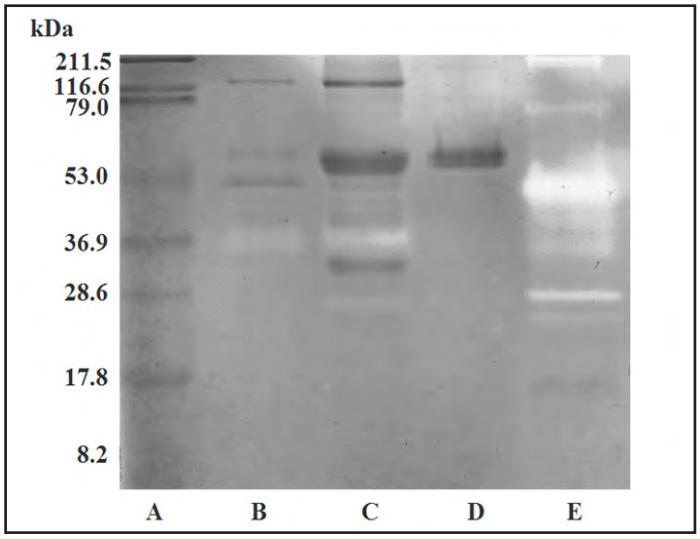

FIGURE 9. GELATINOLYTIC ACTIVITY OF FRACTIONS OF *P.L.H* . A) molecular weight markers; B) 6 µg of *P.l.h* crude venom; C) 6 µg of *P.l.h* FI; D) 6 µg of hut-1; E) 2 µg of *B. colombiensis* crude venom.

In the platelet aggregation assay, hut-1 did not inhibit platelet aggregation in response to the tested agonists (collagen, ADP, ristocetin). This result contrasts with the potent inhibitory action of *P.l.h* crude venom, suggesting that other proteins in this venom induce platelet aggregation [52]

Compared to the titre, which is the volume of serum necessary to neutralize the concentration of bothropic venom [47], as determined by the manufacturer, PAOS had low efficacy at neutralising the haemorrhages induced by the *P.l.h* crude venom, nearly four times the amount recommended by the manufacturer was required. PAOS was even less effective against hut.1 than the crude venom; a concentration 11 times higher than the recommended by the manufacturer was required to neutralise the activity of hut-1 (1 mL of PAOS per 2 mg of venom).

To correlate these results with the antigenic recognition evaluation by Western blot analysis, the antivenom exhibited limited antigenic recognition of some protein bands, specifically, 54, 45 and 30 kDa [FIG.10]. These molecular weights are within the range reported for PII and PI metalloproteases, such as porthidin-1, a 23 kDa haemorrhagic metalloprotease that was previously

isolated from this venom [14]. These observations could indicate that additional metalloproteases exist in the *P.l.h* venom that are not antigenically recognised by the immunoglobulins present in the antivenom and, therefore, are not neutralised. These results markedly contrast the results obtained for hut-1, which was antigenically recognised by PAOS [FIG.10]; however, PAOS did not effectively neutralise the haemorrhagic activity of hut-1, which could be a consequence of the antigen binding at a site that does not corresponding to the activity of hut-1. The ecological area of the specimens whose venom were used corresponds, as detailed in materials and methods, to the Macanao peninsula. It is known that the manufacturer of antivenoms (Biotecfar CA) uses a pool of venoms from specimens that are randomly collected throughout the island, it is also known that venoms have intra-species variability [42] and that geographic variability surely influences the synergistic strategies of predominant toxins components of snake venoms [53].

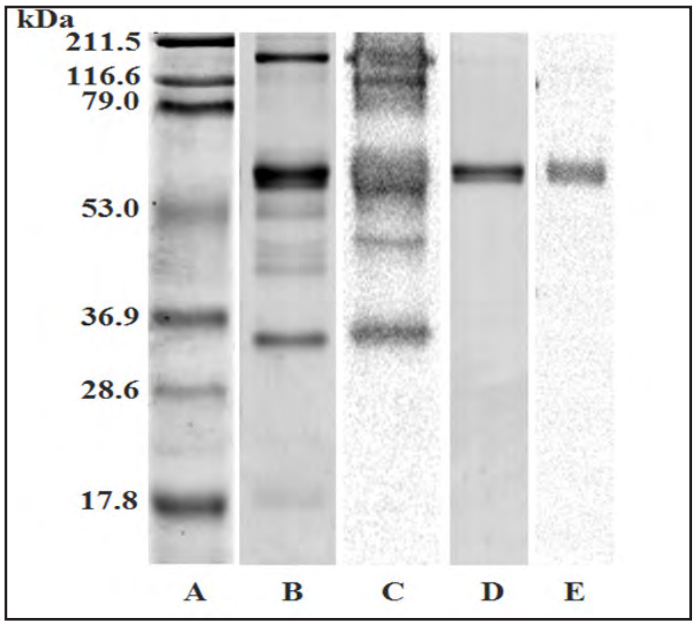

FIGURE 10. WESTERN BLOT PA INTERACTION AGAINST *P.L.H* FI AND HUT-1. A) molecular weight markers; B) SDS-PAGE *P.l.h* FI (10 µg); C) Western blot PA against *P.l.h* FI; D) SDS-PAGE hut-1 (10 µg); E) Western blot PA against hut-1.

CONCLUSIONS

P. lansbergii hutmanni is an epidemiologically important venomous snake species located on Margarita Island (Venezuela). Here, it has been shown that the *P.l.h* venom lacked the marked *in vitro* procoagulant activity characteristic of bothropic venoms, which could have implications in the diagnosis of envenomations considered to be from bothropic snakes. The *P.l.h* crude venom showed very high haemorrhagic and anticoagulant activities, and hut-1, an ~ 62 kDa enzyme classified as a P-III metalloprotease, was identified in this venom. Additionally, hut-1 had a strong fibrinogenolytic and moderate fibrinolytic action and did not exhibit anticoagulant activity. The antivenom PAOS was not able to effectively neutralise the haemorrhagic activity of crude venom, but PAOS did neutralise hut-1; therefore, treatment with this antivenom could have reduced efficacy in the treatment of envenomation by *P.l.h*.

Funding and Acknowledgments

The authors gratefully acknowledge the funding for the research grant (PG: 09-8760-2013), from the Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela, Bolivarian Republic of Venezuela and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) y la Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ-Dr. J. Perales).

Ethical statement

Qualified staff arranged all the experimental methods relating to the use of live animals. These methods were approved by the Institute of Anatomy Ethical Committee of the Universidad Central de Venezuela on 7 March 2018 under assurance number 07-03-18 and followed the norms obtained from the Guidelines for the Care and Use of Laboratory Animals, published by the US National Institute of Health (1985). The research questions asked, the technical methods chosen, and the conclusions reached are exclusively responsibility of the authors.

Conflicts of Interest

The authors declare no conflict of interest.

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EVALUATION OF TRYPANOCIDAL AND ANTHELMINTIC EFFICACY OF AN ISOMETAMIDIUM – IVERMECTIN ASSOCIATION IN BOVINE

EVALUACIÓN DE LA EFICACIA TRIPANOCIDA Y ANTIHELMÍNTICA DE UNA ASOCIACIÓN DE ISOMETAMIDIUM E IVERMECTINA EN BOVINOS

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ABSTRACT

The objective of the present work was to evaluate the efficacy of the compound Hemoveex® (isometamidium chloride 2,4% and ivermectin 2,0%) of Reveex, a Venezuelan laboratory, on the control of mixed infections of *Trypanosoma vivax* and gastrointestinal nematodes in bovine. Sixteen one year old heifers, assigned to two experimental groups (intramuscular (IM) and subcutaneous (SC)), were treated each with Hemoveex® at the dose of 1 mililiter (mL) /50 kg of body weight (BW); previously, all 16 heifers had been inoculated with 5 mL each of bovine blood showing 4 flagellates/100X microscopic field of *Trypanosoma vivax* initially obtained from a cow in Puerto Berrío, Antioquia, Colombia and further multiplied in a calf at the Jaime Isaza Polytechnic of Marinilla, Antioquia. The assignment to either group, was made on the basis of the "strongylid" nematode type egg per gram (epg) of faeces, using the McMaster technique. From day (d) 1 after treatment to d 63, when observations finished, no trypanosomes were seen in any of the sixteen heifers. As for the control of gastrointestinal nematodes, the association showed efficacies of 36, 12, 24 and 84%, on d 7, 28 and 42, and 63 post-treatment (PT), respectively, when applied by the IM route and of 47.8, 39.1 and 78.3%, on d 21, 28 and 63 PT, respectively, when administered (SC). Isometamidium + ivermectin was highly efficacious against *Trypanosoma vivax,* given either IM or SC presented a satisfactory anthelmintic efficacy only by d 63 PT.

Key words: C*attle; control; nematodes; test; trypanosomes*

RESUMEN

El objetivo del presente trabajo fue evaluar la eficacia de la mezcla (cloruro de isometamidio al 2,4% e ivermectina al 2,0%) del laboratorio Reveex de Venezuela, en el control de infecciones por *Trypanosoma vivax* y nemàtodos gastrointestinales en bovinos. Dieciséis bovinos de aproximadamente 1 año de edad divididos en dos grupos, por vía de inoculación intramuscular y subcutánea, fueron tratados con la mezcla isometamidio + ivermectina a la dosis de 1 mililitro (mL)/50 kg peso corporal; antes del tratamiento los 16 animales fueron inoculados vía venosa con 5mL cada uno, de sangre con una parasitaemia de 4 parásitos por campo en frotis de sangre coloreado con Giemsa, de una cepa de *Trypanosoma vivax* adquirida en un bovino de Puerto Berrío, Antioquia, Colombia y multiplicada en un ternero en el Politécnico Colombiano Jaime Isaza Cadavid de Marinilla, Antioquia. La división de los grupos se hizo teniendo en cuenta los huevos de parásitos gastrointestinales tipo "strongylida" por gramo de heces, mediante la técnica de McMaster. Desde un día (d) después del tratamiento, hasta el d 63, cuando finalizaron las observaciones, en ninguno de los 16 animales se observaron tripanosomas. Con respecto al control de parásitos gastrointestinales, el producto mostró eficacias de 36; 12; 24 y 84% en los 17; 28; 42 y 63 post-tratamiento (PT), respectivamente, cuando se aplicó por vía intramuscular (IM) y de 47,8; 39,1 y 78,3% en los d 21; 28 y 63 PT, respectivamente, cuando se aplicó por vía subcutánea (SC). La mezcla isometamidio+ ivermectina resultó altamente eficaz contra *Trypanosoma vivax;* administrada tanto por vía IM; como SC y presentó una eficacia antihelmíntica satisfactoria sólo hacia el d 63 PT.

Palabras clave: C*ontrol; ganado; nematodos; prueba; tripanosomas*

Recibido: 24/03/2020 Aceptado: 10/09/2020

INTRODUCTION

Both, internal (gastrointestinal, pulmonary, hepatic, hematic,etc.) and external (ticks, flies, lice, mites, etc.) cause high economic losses to cattle industry worldwide.

Bovine *(Bos taurus)* trypanosomosis in Africa (caused mainly by Trypanosoma vivax *(T. vivax)* and Trypanosoma congolense), only in the Tse-Tse fly belt, causes losses estimated in United States of America (USA) $ 4,75 billions/year (yr) [18]. In Colombia [5], where the disease is endemic in regions like Inter Andean Valleys, Middle Magdalena, Caribbean Coast and Eastern Plains [6], several studies confirm the economic importance of the disease. Betancourt and Wells [8] recall an episode of trypanosomosis in a dairy in the Cauca Valley where losses went up to USA$ 5,654. Studies conducted in the State of Cordoba in 1996 found that, in three months, calves infected with *T. vivax*, gained an average of 6.0 kg less than non infected calves [1, 34].

Gastrointestinal worms severely affect the productivity of cattle ranches, since it produces anorexia, loss of blood and plasmatic proteins, lung damage, metabolic disturbance, diarrhea, and retarded growth [14, 40].

In South America, the control of *bovine* trypanosomosis has been based for many years on diminazene aceturate and, more recently on isometamidium chloride [11,12].. Both compounds are of common use also in Africa [24, 25, 29] and are marketed as single molecules. Control of gastrointestinal parasitism (GIP) is mainly done with benzimidazol derivatives (albendazol, fenbendazol and others), imidazotiazols (levamisol, tetramizol), macrociclic lactones (Ivermectinas, milbemicines), organophosphates (haloxon, triclorfon). All of them are also sold as single molecules. [13, 27, 33].

The colombian veterinary market does not have a compound containing both, isometamidium and ivermectin. Such a product would be useful, considering that a high worm burden produces immunodepression and could complicate a trypanosomosis clinical episode. Reveex Laboratory has developed a mixed product containing both drugs. It is expected that the product, while controlling gastrointestinal parasitism, favors the preventive and curative effect on trypanosomes. The present work was conducted to evaluate the efficacy of isometamidium and ivermectin combination, on the control of mixed infections by gastrointestinal parasites (GIP) and *T. vivax* in cattle.

Trypanosomosis

Bovine trypanosomosis produced by T. vivax is known in Colombia since 1931 [37, 49] and it is considered endemic in regions like: the Atlantic Coast, Cauca and Magdalena River Valleys and the Eastern Planes [6, 22, 34, 46] in warm zones and under 1500 meters above sea level (m.a.s.l). Recently the presence of the parasite was reported in cattle in Antioquia, at 2.486 m.a.s.l. [50]. The disease has a strong economic impact due to abortions, anaemia, reduction of milk yield and control costs. [3, 4, 8, 9, 16, 34, 47].

Gastrointestinal parasites

Most known nematode genera have been reported in association with GIP in cattle in Colombia. In the Eastern Planes, Cooperia, Haemonchus, Ostertagia, Oesophagostomum, Trichostrongylus, Trichuris, Bunostomum, Capillaria, Agriostomum, Toxocara and Mecistocirrus have been found by different workers [32, 35, 39, 44]. In the Atlantic Coast, the genera Strongyloides, Toxocara, Cooperia, Haemonchus, Mecistocirrus, Oesophagostomum, Bunostomum, and Ostertagia have been registered in Cordoba State and the genera Strongyloides, Haemonchus, and Trichostrongylus in Cesar State [36, 41].

In the Middle Magdalena the genera Strongyloides, Cooperia, Ostertagia, Haemonchus, Oesophagostomum, Trichostrongylus and Bunostomum have also been found [13]. Another study conducted in Santander State, found that the most common nematodes infecting bovine in the García Rovira Province were Toxocara, Cooperia, Haemonchus, Ostertagia, Nematodirus and Trichostrongylus [38].

Generally speaking, GIP is more prevalent and severe in young calves. Villar and Arguelles [43], found the highest counts of eggs in faeces in calves 105 to 130 days (d). Some genera, like Toxocara and Strongyloides, are more common in younger calves [41, 44].

As for anthelmintics used in controlling GIP worms in bovine, studies in a milk producing area of Boyacá, found that Albendazol was the most commonly used (30%) [47], followed by ivermectin (14%) and levamisol (7%) [33]. Another work reported the oral use of 1% diatomaea sands as anthelmintic for cattle and reported 84 and 100% reduction in egg per gram (epg) of faeces count on d 90 and 135, respectively [28]. Marquez *et al.* [30], reported anthelmintic resistance in 25% of the farms examined at the Altiplano Cundiboyacense region. Resistance to albendazol and ivermectin was reported on 17 and 8% of these farms, respectively.

MATERIALS AND METHODS

The study was conducted at the Román Gómez Farm of the Jaime Isaza Cadavid Polytechnic in Marinilla, Antioquia located at 6º11’47” North; 75º 20´ 0” West.

The drug tested: The Hemoveex® (Reveex Laboratories, Venezuela), compound tested on its trypanocidal and anthelmintic efficacy, is an association of isometamidium chlorhidrate 2,4% and ivermectin 2,0%. Ivermectin is an endectocide known for over 30 years. It works by stimulating the presynaptic GABA relaxation with the resultant blockage of the nervous impulse in the post-

synaptic transmission, leading to paralysis and death of the parasite. According to Gregorio's description, ivermectin binds to Cl ions regulated by glutamate (GluCl) located in muscular and nervous cells of invertebrates. This interaction leads to an increase in the permeability to Cl- causing membrane hyperpolarization with paralysis and death of the parasite [21]. As for isometamidium, in the market for more than 50 yr, its mode of action is not fully understood. It is believed that selectively inhibits the kinetoplastic II topoisomerase of the trypanosome [10, 26]. Isometamidium also blocks nucleic acid synthesis [45]. Ivermectin and isometamidium have very different loci of action and work on very different processes; this discards any possible interaction between them.

Trypanosoma vivax strain: A *T. vivax* working strain was originally obtained from a natural infection in a cow at Puerto Berrío, Antioquia and kept in liquid nitrogen at the CES, Colombian Institute of Tropical Medicine.

Gastrointestinal nematodes: The study was performed using the natural nematode infections present in the calves when obtained for the study. Worm burdens were estimated on the basis of epg of faeces count using the McMaster Technique as described by Dunn [17]. Segmented eggs were named "strongylid" type, a term that includes eggs of the genera Haemonchus, Trichostrongylus, Ostertagia, Cooperia, Mecistocirrus, Nematodirus, Bunostomum and Oesophagostomum given the difficulty in telling them apart.

Experimental animals: Sixteen one year old heifers, *Bos taurus* crosses, with an average weight of 140 kilograms (kg) were obtained, kept stabled in the farm's facilities and fed fresh chopped grass, hay and commercial ration (2 kg/animal/d), salt and water *ad libitum.*

Inoculations with *T. vivax*: A heifer (Num. 0054) was treated on three consecutive d, with dexamethasone by the ntramuscular (IM) route, at a dose rate of 0.1 miligrams (mg) /kg of body weight, and then inoculated intravenously (IV) with 5 mililiters (mL) of blood containing the working strain of *T. vivax* with a parasitaemia of 4.5 flagellates/100 X microscopic fields using an Olympus CX31 Japan equipment, as seen in a thin smear stained with Giemsa. From here onward, the blood of the heifer was examined daily or every other day to look for trypanosomes (in 50 microscopic fields of a thin smear) and to determine the haematocrit value. Body temperature was also measured at the same time. Once a high parasitaemia was observed, the reamining 15 heifers were inoculated IV, with 5 mililiters (mL) each of blood from heifer 0054, and monitored as described to determine clinical (temperature), haematological (haematocrit) and parasitologic (parasitaemia) values. Once parasitaemia with *T. vivax* was evident, all heifers were treated, either IV or IM, with Hemoveex®, at a dose rate of 1mL/50 kg BW, and monitored as described, on d [3, 7, 14, 21, 28, 42 and 63] post-treatment (PT).

Gastrointestinal parasites (GIP): On the same day of treatment with isometamidium + ivermectin, all heifers were examined for gastrointestinal nematodes, using the McMaster technique. The heifers were assigned to either SC or IM group, depending on the route by which the compound would be applied. On the same d, a pool of faeces from those heifers showing the highest epg counts was submitted to cultivation by the Corticelli and Lai technique as described by Niec [31], to obtain L3 nematode larvae for further genera identification. Monitoring of GIP was done on the same PT d, described for *T. vivax*, and expressed in terms of epg of the "strongylid" type.

Efficacy: The efficacy of treatments was estimated as percentage parasitaemia and epg on each PT d, as compared with pretreatment values for the same variables.

Weight gain: Body weight for each heifer was measured both, at the beginning and at the end of the study.

Statistical analysis Temperature, parasitaemia and haematocrit values, as well as eggs per gram (epg) and body weight data, before and after treatment, were compared by using the ANOVA test (analysis of variance), and accepting a significance level of 0,05 (5% error). All statistical calculations were made with the aid of the STATA program [2].

RESULTS AND DISCUSSION

Trypanosomes in donor heifer 0054

On d 11 post-inoculation, heifer 0054 presented a haematocrit of 26%, a body temperature of 39,0ºC and a parasitaemia of 4,5 trypanosomes/field in a blood-stained smear. On this d, blood was collected with Ethylene Diamine Tetraacetic Acid (EDTA) as anticoagulant to inoculate the 15 remaining experimental bovines. Each heifer received IV 5 mL of parasitemic blood and was assigned to either the IM or the SC group.

Trypanosomes in the experimental heifers

On d six after inoculation, all heifers showed parasitaemia with *T. vivax.* The IM group, presented an average parasitaemia of 2.6 trypanosomes/field and the SC group presented an average parasitaemia of 5 trypanosomes/field on stained blood smears. The incubation period observed for *T. vivax* in the present work, was similar to that reported by different researchers in Africa and America [3, 6, 15, 23], especially when the infection is the result of IV inoculation.

On d 7 PT, eight heifers were treated IM and the remaining eight heifers SC, with isometamidium + ivermectin association at a dose rate of 1mL/50 kg of body weight. From d 1 PT to d 63 PT, no trypanosomes were seen in any of the experimental heifers with the Woo´s [48] and Giemsa stained blood parasitological techniques employed. Parasitaemia readings on d 0 (treatment day) and until d 63 PT are presented on TABLES I and II, for the

TABLE I

***Trypanosoma vivax* PARASITAEMIA IN HEIFERS TREATED WITH ISOMETAMIDIUM – IVERMECTIN BY THE INTRAMUSCULAR (IM) ROUTE**

	Trypanosomes (N x microscopic field)							
Identification	Experimental Days							
Animal Number	0	3	7	14	21	28	42	63
168	3,6	0	0	0	0	0	0	0
185	3,0	0	0	0	0	0	0	0
194	4,0	0	0	0	0	0	0	0
190	1,0	0	0	0	0	0	0	0
177	0,8	0	0	0	0	0	0	0
182	1,4	0	0	0	0	0	0	0
054	4,5	0	0	0	0	0	0	0
180	2,5	0	0	0	0	0	0	0
Total	20,8	0	0	0	0	0	0	0
Average	2,6	0	0	0	0	0	0	0

TABLE II

***Trypanosoma vivax* PARASITAEMIA IN HEIFERS TREATED WITH ISOMETAMIDIUM-IVERMECTIN BY THE SUBCUTANEOUS ROUTE**

	Trypanosomes (N x microscopic field)							
Identification	Experimental Days							
Animal Number	0	3	7	14	21	28	42	63
183	13,0	0	0	0	0	0	0	0
187	1,6	0	0	0	0	0	0	0
009	15,0	0	0	0	0	0	0	0
186	3,8	0	0	0	0	0	0	0
179	0,1	0	0	0	0	0	0	0
189	2,0	0	0	0	0	0	0	0
192	2,5	0	0	0	0	0	0	0
175	2,2	0	0	0	0	0	0	0
Total	40,2	0	0	0	0	0	0	0
Average	5,0	0	0	0	0	0	0	0

IM and SC groups, respectively.

Efficacy

The absence of trypanosomes in the blood of all heifers from d 1 to d 63 PT, both in the IM and SC groups, demonstrated that under the conditions of the present study the association was 100% efficacious in controlling *T. vivax* infections in bovine, at least until d 63 PT. The early, 24 (h) curative effect provided by isometamidium has also been reported [42] and its efficacy as preventive and curative of infections with the parasite, as well as its long lasting protection, up to six months has been previously documented corchetes, [8, 11, 12, 15, 19, 20, 24, 29, 42].

Temperature readings

In the IM group, on the d of administration, being *T. vivax* present, five, out of eight heifers presented a body temperature ≥ 39ºC, with an average for the group of 39.2ºC. From here on, averages of body temperature for the group were 38.8, 38.5, 38.3, 38.6, 38.8, 38.5 and 39.0ºC for the d 3, 7, 12, 14, 19, 21, 28, 42 and 63 PT, respectively (FIG 1).

n the SC group, on the day of the administration, being *T. vivax* present in all heifers, seven of eight animals had a body temperature ≥ 39.1ºC, with an average for the group of 39.4ºC. From here on, except for two heifers on d 3 PT, and one with wild behaviour

which was always hyperthermic, body temperature was normal, with averages of 38.7, 38.2, 38.4, 38.6, 38.8, 38.4 and 38.7ºC on d 3, 7, 14, 21, 28, 42 and 63 PT, respectively (FIG. 1).

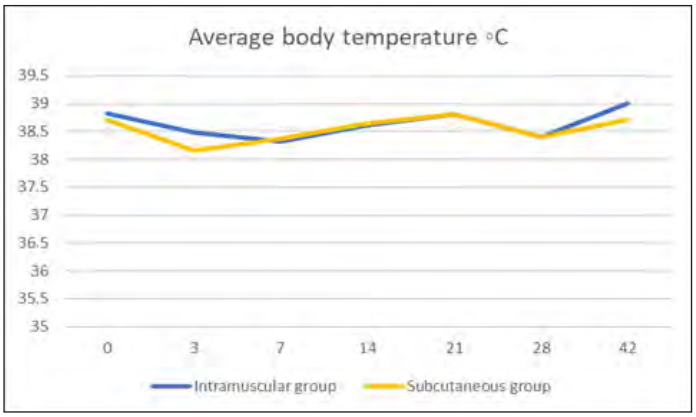

FIGURE 1. AVERAGE BODY TEMPERATURE ON HEIFERS TREATED WITH ISOMETAMIDIUM + IVERMECTIN BY INTRAMUSCULAR OR SUBCUTANEOUS ROUTE.

Haematocrit values

In the IM group, on the d of the administration, haematocrit values were normal, with an average for the group of 36.9%. From here on, the haematocrit increased until d 63 PT, with averages of 38.4, 45.4, 47.2, 50.6, 42.9, 45.2 and 49.1% for d 3, 7, 14, 21, 28, 42 and 63 PT, respectively (FIG. 2).

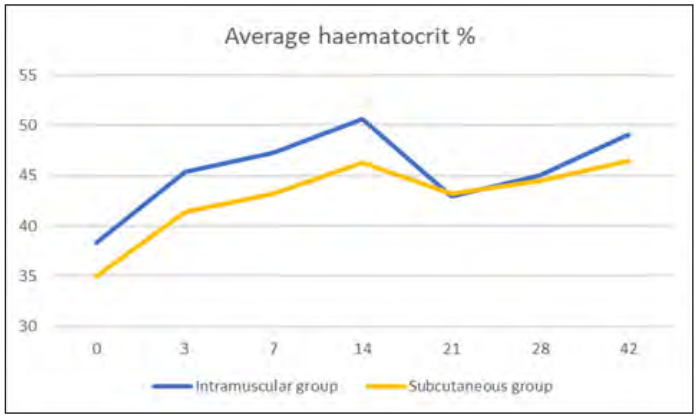

FIGURE 2. AVERAGE HAEMATOCRIT VALUES IN HEIFERS TREATED WITH ISOMETAMIDIUM + IVERMECTIN BY INTRAMUSCULAR OR SUBCUTANEOUS ROUTES.

In the SC group, on the d of treatment with isometamidium-ivermectin, the haematocrit values were normal, with an average for the group of 34.7%. From here on, the haematocrit increased reaching average values of 35.0, 41.4, 43.2, 46.2, 43.2, 44.5 and 46.5% for d 3, 7,14, 21, 28, 42 and 63 PT, respectively (FIG. 2).

Gastrointestinal parasitism

Although early in the study Moniezia eggs and Eimeria oocysts were seen in some animals; they were not included in the results, since isometamidium + ivermectin has no effect on this type of parasites.

"Strongylid" type egg counts in both treated groups are presented in TABLES III and IV and FIG. 3. In the IM group, initial epg counts averaged 312.5. In terms of efficacy, the compound showed reduction of GIP on d 7, 28, 42 and 63 PT, the average efficacies being 36, 12, 24 and 84%, respectively. On d 14 PT, there was a marked increase in the epg values for the group, possibly due to eggs laid by adult worms that were on the larval stage in the intestinal mucose, on the day of the treatment (TABLE III).

Nematode eggs obsrvedin the feces ofheifers in the presnt work, were al lof the "strongylid" type, which includes many of the genera reported by other workers [5,13,32,35,36,38,41,44]

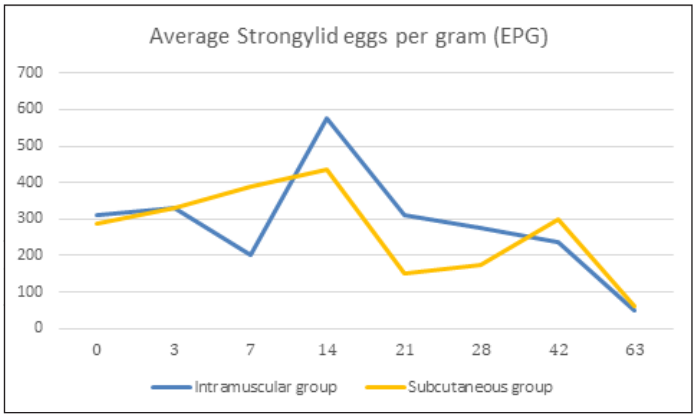

FIGURE 3. AVERAGE "STRONGYLID" TYPE EGGS PER GRAM OF FAECES IN HEIFERS TREATED WITH HEMOVEEX® BY INTRAMUSCULAR OR SUBCUTANEOUS ROUTE.

Cultivation of faeces

Cultivation of faeces yielded no L3 nematode infective larvae. For this reason, identification of nematode genera was not possible.

Statystical analysis

ANOVA test [2] did not show statistical ($P<0.05$) difference between IM and SC groups for the variables: parasitaemia with T. vivax, body temperature, haematocrit, nematode egg counts and weight gain.

TABLE III
NUMBER OF STRONGYLID TYPE EGGS PER GRAM OF FAECES IN HEIFERS TREATED WITH ISOMETAMIDIUM-IVERMECTIN BY THE INTRAMUSCULAR ROUTE

	Strongylid type (epg)							
Identification	**Experimental Days**							
Animal number	**0**	**3**	**7**	**14**	**21**	**28**	**42**	**63**
168	700	400	200	200	300	200	300	50
185	400	400	300	1,600	800	500	100	50
194	100	250	200	400	300	200	200	50
190	350	50	300	1,400	200	500	300	50
177	250	0	100	0	0	100	100	0
182	350	400	200	500	500	200	600	0
054	100	250	100	100	200	200	300	150
180	250	900	200	400	200	300	0	50
Total	2500	2650	1600	4600	2500	2200	1900	400
Average	312,5	331,25	200	575	312,5	275	237,5	50
Efficacy %	-	0	36	0	0	12	24	84

TABLE IV
NUMBER OF STRONGYLID TYPE EGGS PER GRAM OF FAECES IN HEIFERS TREATED WITH ISOMETAMIDIUM - IVERMECTIN BY THE SUBCUTANEOUS ROUTE

	Strongylid type (epg)							
Identification	**Experimental Days**							
Animal number	**0**	**3**	**7**	**14**	**21**	**28**	**42**	**63**
183	550	550	400	700	300	200	400	150
187	100	0	600	500	0	200	300	0
009	350	350	600	500	300	100	400	250
186	0	600	300	200	0	100	100	0
179	50	250	300	100	200	200	500	100
189	350	300	500	500	0	100	100	0
192	650	400	200	700	300	200	300	0
175	250	200	200	300	100	300	300	0
Total	2300	2650	3100	3500	1200	1400	2400	500
Average	287,5	331,25	387,5	437,5	150	175	300	62,5
Efficacy %	-	0	0	0	47,8	39,1	0	78,3

Undesirable reactions

No systemic or local undesirable reactions were detected in any of the heifers treated with the association, neither on the d. of treatment, nor on any of the post treatment d.

Weight gain

TABLES V and VI present the wight values for each heifer at the beginning and the end of the study in the IM and SC groups, respectively. Confinement and suffering clinical tripanosomosis did not seem to have severely affected weight gain. In the IM group, average weight gain was 43.6 kg whilst in the SC group, it was 42.1 kg, during the 63 d of the study.

CONCLUSIONS

Isometamidium + ivermectin association was 100% efficacious in controlling *T. vivax* infections in cattle from the days following IM or SC administration to d 63 PT.

Body temperature and haematocrit values significantly improved in all trypanosome infected bovine after treatment.

TABLE VI
INITIAL AND FINAL BODY WEIGHT VALUES IN HEIFERS TREATED WITH ISOMETAMIDIUM - IVERMECTIN BY THE SUBCUTANEOUS ROUTE

Animal Number	Initial Body Weight	Final Body Weight
183	181	239
187	138	190
009	120	150
186	148	168
179	150	179
189	136	174
192	144	216
175	148	186
Total	1,165	1,505
Average	145,6	187,7

In the first 21 d PT with the association given IM and 14 d PT given SC, anthelmintic efficacy of the compound was low, but then increased by d 63 PT and reached values of 84 and 78.3%, respectively.

The study did not reveal any difference in efficacy against trypanosomes and GIP nematodes between heifers treated by the IM or the SC route.

It seems that isometamidium + ivermectin, prevented weight loss in bovine suffering a clinical episode of tripanosomosis and a simultaneous infection with GIP nematodes. All treated heifers had gained weight significantly during the duration of the study.

The association did not cause any local or systemic undesirable reactions in bovine during the 63 d following its administration either by the IM or the SC route.

ACKNOWLEDGMENTS

The excellent collaboration of the staff and operative support personnel of the Jaime Isaza Cadavid Polytechnic in the Marinilla Farm and the finantial and logistic support of Reveex, are highly appreciated.

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FE DE ERRATA

CARACTERIZACIÓN DE POLIMORFISMOS DEL GEN LEPTINA EN SEMENTALES DE LA RAZA CARORA

Characterization of Leptin gene polymorphisms in Carora sires

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TABLA IV
FRECUENCIAS ALÉLICAS Y GENOTÍPICAS DE LOS POLIMORFISMOS DEL GEN LEP EN TOROS DE RAZA CARORA

Polimorfismo	Frecuencias alélicas		Frecuencias genotípicas	
rs29004487	A (84)	0,98	AA (41)	0,95
	T (2)	0,02	TT (2)	0,05
rs29004501	C (69)	0,8	CC (29)	0,67
			CT (11)	0,26
	T (17)	0,2	TT (3)	0,07
rs29004488	T (51)	0,59	CC (6)	0,14
			CT (23)	0,53
	C (35)	0,41	TT (14)	0,33
rs29004508	C (79)	0,94	CC (37)	0,88
	T (5)	0,06	CT (5)	0,12

***** En la pag. 88 del Vol.XXX (2) 2020 se sustituye la tabla *TABLA IV* , por esta nueva.

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Vol, XXX, Nº 3

Esta revista fue editada en formato digital y publicada en Diciembre 2020, por ***La Facultad de Ciencias Veterinarias, Universidad del Zulia. Maracaibo-Venezuela.***

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Nuestro objetivo es informar a los autores de la decisión sobre sumanuscrito en unplazo de ocho semanas de suentrega. Tras la aceptación de su artículo, el mismo será publicado en el número inmediatamente disponible.

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Esa sí que en nombre del comité editor, le solicito su apoyo para que esta iniciativa continúe, mediante la publicación de sus documentos en esta revista.

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8. **Conclusiones:** Las mismas deben ser claras y precisas en ellas no se aceptan llamados a Referencias Biográficas

9. **Agradecimiento.** Sólo se aceptan agradecimientos institucionales.

10. **Referencias Bibliográficas**. Deben presentarse en una lista numerada y en estricto orden alfabético. Deben ser citadas en el texto (en orden cronológico) usando números arábigos entre corchetes. Estos números deben parearse con los de la lista de referencias bibliográficas. No se aceptan como referencias trabajos no publicados, artículos de periódicos o revistas populares ni comunicaciones personales. Evite el uso excesivo de literatura no arbitrada (e.g., libros, manuales, referencias electrónicas en exceso, etc.). No deben citarse resúmenes, a menos que sean la única referencia en relación a un aspecto importante. Los autores son responsables del uso correcto y presentación de las referencias. No se permiten citas bibliográficas en el Resumen, abstract ni en Conclusiones, en Tablas y Figuras. Los siguientes son ejemplos recomendados:

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[2] FARIA, M.H.; TONHATI, H.; NADER-FILHO, A.; DUARTE, J.M.C. Milk production and some constituents in two buffalo herds in São Paulo State, Brazil. **Proceeding 5th World Buffalo Congress.** Caserta, 10/13-16. Pp 30-38. 1997.

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6. **Tables.** These should be appropriately cited in the text and must be identified and consecutively numbered using Roman numerals. A concise and descriptive heading is requested. Abbreviations or symbols should be explained in a caption below the table. Title must be above the table in capital letters. Title should be presented in both, in Spanish or English, depending of the language in which the work was written, no simultaneously. Xerox copies will not be accepted Keep tables as simple as possible. Tables are self-contained with the title and footnotes providing all information to understand the table without referring the text separated with semicolon. Tables should be included in the text immediately after mentioning them.

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[1] LEELAND, W.W. Steady state kinetics. In: **The Enzymes.** Boyer, P. (Ed.). 2nd Ed. Academic Press, New York. 66 pp. 1970.

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[2] FARIA, M.H.; TONHATI, H.; NADER-FILHO, A.; DUARTE, J.M.C. Milk production and some constituents in two buffalo herds in São Paulo State, Brazil. **Proceeding 5th World Buffalo Congress.** Caserta, 10/13-16. Italy. 140 pp 1997.

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