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SEMILLA DE LUPINO (*Lupinus angustifolius* L.) DESCASCARADA EN DIETAS CON ENZIMAS PARA POLLOS DE ENGORDA

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SEMILLA DE LUPINO (*Lupinus angustifolius* L.) DESCASCARADA EN DIETAS CON ENZIMAS PARA POLLOS DE ENGORDA

Fredy Mera Zúñiga, Dr.

Colegio de Postgraduados, 2018

RESUMEN

El valor alimenticio de la semilla de lupino (*Lupinus angustifolius* variedad Boregine) descascarada, producida en Zapopan, Jalisco, México, para pollos de engorda se evaluó en términos de su composición química, energía metabolizable aparente y sus efectos en el comportamiento productivo, tamaño de órganos digestivos, indicadores de bienestar animal y digestibilidad ileal de la dieta. Tres experimentos se llevaron a cabo en las instalaciones avícolas del Colegio de Postgraduados, Campus Montecillo, el cual se ubica en el municipio de Texcoco, Estado de México, a 19° 29’ N y 98° 54’ O, a una altitud de 2,247 m.

Los resultados del primer experimento reportado en el Capítulo 2 muestran que el descascarado de las semillas de lupino incrementó los valores de energía metabolizable aparente, lo cual puede atribuirse a la disminución de las fracciones de fibra de estas semillas después del descascarillado. La eliminación de las cáscaras también aumentó las concentraciones de aminoácidos, proteína y extracto etéreo. Con base en estos resultados, se sugiere que la semilla de lupino descascarada es una mejor alternativa que la semilla entera para reemplazar a la pasta de soya en dietas para aves, en vista de la mayor concentración de aminoácidos, proteína y energía metabolizable.

Los efectos de la alimentación con dietas donde se reemplazó totalmente a la pasta de soya por semilla de lupino descascarada (SLD) y suplementadas con enzimas (xilanasas, proteasas y amilasas) en el comportamiento productivo, el tamaño de órganos digestivos e indicadores de bienestar de pollos de engorda se investigaron en el segundo experimento incluido en Capitulo 2. Los resultados mostraron que el peso final y la ganancia de peso de los pollos alimentados con dietas con SLD y suplementadas con enzimas fueron similares a la de los pollos de la dieta testigo (dieta a base de maíz-pasta de soya sin enzimas), pero el consumo de alimento y la conversión alimenticia fueron mayores. En este experimento, se observó un incremento en el peso relativo del proventrículo y molleja en los pollos alimentados con dietas con SLD, lo cual se debió probablemente al mayor contenido de polisacáridos no amiláceos de la dieta. Las dietas con SLD también deterioraron las variables de bienestar (menor habilidad para caminar y latencia postrarse y mayor angulación de las patas), lo cual posiblemente esté influenciado por la mayor humedad de las excretas que afectó la calidad de la cama.

En el experimento final (Capítulo 3), se evaluó el efecto de la sustitución total de pasta de soya por SLD y la suplementación de enzimas (B-glucanas, xilanasas y proteasas) en dietas de iniciación para pollos de engorda sobre la digestibilidad ileal aparente de la materia seca, materia orgánica, proteína y extracto etéreo, así como el valor de energía metabolizable aparente corregida por nitrógeno (EMAn) de la dieta. Se encontró que la sustitución de pasta de soya por SLD disminuye de digestibilidad ileal aparente de la materia seca, materia orgánica y extracto etéreo y el valor de EMAn de la dieta. Sin embargo, la digestibilidad ileal aparente de la proteína fue ligeramente, pero significativamente mayor, en las dietas con SLD, lo que indica que la proteína de la SLD es tan digerible o más que la proteína de la pasta de soya. Debido al menor valor de EMAn y la menor digestibilidad de la dieta con SLD se disminuyó la ganancia de peso de los pollos e incrementó la conversión alimenticia. La suplementación de enzimas incrementó el valor de EMAn y la ganancia de peso, pero sólo en los pollos alimentados con las dietas a base de maíz-pasta de soya, mientras que en las dietas a base de maíz-SLD no se observó ningún efecto significativo en la digestibilidad ni en el valor de EMAn.

Palabras clave: Leguminosas; Tamaño de Órganos Digestivos; Energía Metabolizable Aparente; Digestibilidad Ileal; Variables de bienestar.

DEHULLED LUPINE (*Lupinus angustifolius* L.) SEED IN DIETS WITH ENZYMES FOR BROILERS

Fredy Mera Zúñiga, Dr.

Colegio de Postgraduados, 2018

ABSTRACT

The nutritional value of the dehulled lupine (*Lupinus angustifolius* cv. Boregine) seed, produced in Zapopan, Jalisco, Mexico, for broiler chickens was evaluated in terms of its chemical composition, apparent metabolizable energy and the effects on productive performance, size of digestive organs, welfare-related variables and ileal digestibility of the diet. Three experiments were carried out in the poultry facilities of the Colegio de Postgraduados, Campus Montecillo, located in Texcoco, State of Mexico, at 19° 29 'N and 98° 54' W, and an altitude of 2,247 m.

The results of the first experiment, reported in Chapter 2, show that dehulling of the lupine seeds increased the values of apparent metabolizable energy, which may be attributed to the decrease in fiber fractions of these seeds after dehulling. The removal of hulls also increased the amino acids, protein and ether extract concentrations. Based on these results, it is suggested that the dehulled lupine seed would be a better alternative than whole seed to replace soybean meal in poultry diets in view of the higher concentration of amino acids, protein and metabolizable energy.

The effects of feeding diets, where soybean meal was completely replaced by dehulled lupine seed (DLS) and supplemented with enzymes (xylanases, proteases and amylases) on productive performance, size of the digestive organs and welfare-related variables of broiler chickens were investigated in the second experiment included in Chapter 2. The results showed that the final weight and weight gain of chickens fed diets containing DLS and supplemented with enzymes were similar to that of the control diet (diet based on maize-soybean meal without enzymes), but feed intake and feed conversion were higher. In this experiment, an increase in the relative weight of the proventriculus and gizzard was observed in chickens fed diet containing DLS, which was probably due to the non-starch polysaccharide content of the diet. Diets containing DLS also deteriorated the welfare-related variables (poor walking ability, less latency to lie down and more leg angulation), which is possibly influenced by the higher excreta moisture that affected the litter quality.

In the final experiment (Chapter 3), the effects of the total substitution of soybean meal by DLS and the supplementation with enzymes (B-glucanases, xylanases and proteases) in starter diets for broilers on apparent ileal digestibility of dry matter, organic matter, protein and ether extract, and the apparent metabolizable energy value corrected by nitrogen (AMEn) of the diet, were evaluated. It was found that the substitution of soybean meal by DLS decreases the apparent ileal digestibility of dry matter, organic matter and ether extract and the AMEn value of the diet. However, the apparent ileal digestibility of the protein was slightly, but significantly higher in diets with DLS, indicating that the DLS protein is as digestible or more as soybean meal protein. Due to the lower AMEn value and the lower digestibility of the diet containing DLS, the weight gain of the chickens decreased and the feed conversion increased. Enzyme supplementation increased AMEn value and weight gain, but only in chickens fed diets based on maize-soybean meal, whereas in diets based on maize-DLS no significant effects were observed in digestibility or in the AMEn value.

Key words: Legume; Apparent Metabolizable Energy; Digestive Organ Size; ileal digestibility; Welfare-Related Variables.

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INTRODUCCIÓN GENERAL

En México la avicultura es una actividad importante; entre las carnes la de pollo es la de mayor consumo y producción. En 2017 el consumo per cápita de carne pollo fue de 32.24 kg y la producción de casi 3.5 millones de toneladas, con aumentos a una tasa de anual del 4%. Esto implica mayor demanda de ingredientes alimenticios para la elaboración de las dietas para estas aves, lo que se constata en el incremento del consumo de insumos agrícolas que de 1994 al 2017 fue de 82% (UNA, 2018). De los ingredientes utilizados, las fuentes de proteína son el segundo componente más importante de las dietas para aves y son generalmente más caras que las fuentes de energía (Iji et al., 2017). En México la principal fuente de proteína para la producción avícola es la pasta de soya, subproducto de la industria aceitera; sin embargo, el frijol soya no se produce en cantidades suficientes y el 96% se importa principalmente de Estados Unidos (Martinez-Melendez y Bennett, 2016). Debido al número limitado de países productores y la alta demanda, los precios de la pasta de soya tienden a fluctuar con los cambios en las condiciones climáticas y las situaciones sociales en los países donde se produce la soya (Estados Unidos, Brasil y Argentina), por lo que productores de muchos países que no la producen, exploran fuentes alternativas de proteína para sus industrias (Iji et al., 2017).

La semilla de lupino como fuente de proteína alternativa en dietas para aves de corral ha recibido una atención creciente en países donde las condiciones edafo-climáticas no son adecuados para la producción de soya (Kaczmarek et al., 2016). *Lupinus* es un género diverso de la familia de las leguminosas. Las cuatro especies de lupino que tienen importancia agrícola son: *L. angustifolius*, *L. albus*, *L. luteus* y *L. mutabilis* (Nadal *et al*., 2004). La semilla de *L. angustifolius* es uno de los ingrediente principales en dietas para la alimentación animal en Australia, Japón y Corea, debido a su contenido de proteína de 35% en base seca y de aceite de 5 a 6% (Petterson, 2000). Sin embargo, no se encontraron reportes de producción, comercialización y utilización de lupino en México (SIAP, 2015). El lupino en México sólo se ha cultivado de manera experimental, recientemente Natera et al. (2017) evaluaron seis cultivares de *Lupinus angustifolius* durante dos años en suelos ácidos en Zapopan, Jalisco, México, y reportaron que el rendimiento de grano varió de 2850 a 5016 kg por ha, por lo que sugieren que el cultivo de lupino en México puede alcanzar rendimientos comparables a los alcanzados en áreas donde el lupino es un cultivo tradicional. En este sentido una alternativa importante para evitar la dependencia de la soya en México, podría ser la incorporación al cultivo de especies introducidas como el lupino que tiene potencial de rendimiento y características nutricionales para la alimentación animal.

En el pasado, el uso de semilla de *Lupinus angustifolius* como fuente de proteína para aves de corral era limitado debido al alto contenido de alcaloides que afectan negativamente el crecimiento, consumo de alimento y utilización de nutrientes (Hughes et al., 2000). Sin embargo, durante las últimas décadas, los fitomejoradores han desarrollado variedades de lupino con bajo contenido de alcaloides que son adecuados para la alimentación animal (Degola y Jonkus, 2018). Actualmente, la concentración de polisacáridos no amiláceos (PNA) que es casi dos veces más alta en los lupinos que en otras fuentes de proteína vegetal (Bach Knudsen, 1997) es el factor que restringe el uso de esta semilla en las dietas para aves. Esto se debe a que no hay enzimas endógenas que degraden los PNA en el sistema digestivo de las aves (Smulikowska et al., 2014) y se considera que los PNA del lupino afectan la utilización de nutrientes del alimento (Konieczka y Smulikowska, 2018).

Nalle et al. (2011) sugieren que la remoción de la cáscara y el uso de enzimas exógenas permitiría incluir cantidades altas de semilla de lupino en las dietas para pollo de engorda. Se han realizado estudios para evaluar el efecto de la suplementación de enzimas exógenas en dietas a base de semilla de *Lupinus angustifolius* para aves; sin embargo, la mayoría de los experimentos se realizaron usando semilla entera (Smulikowska et al., 2014) y en menor número usando semilla de descascarada (Annison et al., 1996; Hughes et al., 2000). Los resultados han sido variables, algunos autores no reportan ningún efecto, mientras que otros indican un aumento significativo en digestibilidad, energía metabolizable aparente (EMA) y productividad (Hughes et al., 2000; Steenfeldt et al., 2003). El efecto del descascarado también ha sido reportado por Nalle et al., (2010) quienes indicaron que la remoción de la cáscara en semillas de lupino disminuye el contenido de PNA, e incrementa el contenido de EMA y las concentraciones de aminoácidos.

Con base en lo anterior, la pregunta de investigación que se abordó fue: ¿Es posible sustituir totalmente la pasta de soya por semilla descascarada de lupino en dietas suplementadas con enzimas sin afectar la digestibilidad, el valor de EMA, el comportamiento productivo, el tamaño de órganos digestivos y las variables de bienestar de pollos de engorda? Para responderla, se realizaron tres experimentos con los siguientes objetivos específicos:

1. Caracterizar el contenido de nutrientes, en términos de componentes proximales, incluyendo fibra, calcio y fósforo y perfil de aminoácidos de la semilla entera y sin cáscara de lupino (*Lupinus angustifolius* variedad Boregine).

2. Determinar la energía metabolizable aparente de la semilla entera y sin cáscara de lupino (*Lupinus angustifolius* variedad Boregine) para pollos de engorda.
3. Evaluar el comportamiento productivo, desarrollo de sistema digestivo y variables de bienestar animal de pollos de engorda alimentados con dietas donde se reemplazó totalmente la pasta de soya por semilla descascarada de lupino suplementadas con enzimas.
4. Determinar el efecto de la semilla descascarada de lupino y la suplementación de enzimas en la digestibilidad ileal y valor de energía metabolizable de la dieta en pollos de engorda en etapa de iniciación.

CAPÍTULO I

REVISIÓN DE LITERATURA

1.1. El cultivo de Lupino

Las leguminosas son cultivos multipropósito, su grano sirve como alimento para humanos y para animales, con un alto valor nutricional como fuente de proteína. La capacidad de asimilación de nitrógeno atmosférico realizado por bacterias (*Rhizobium* spp.) que viven en simbiosis con las leguminosas (Nadal et al., 2004) hace que estas plantas sean importantes en los sistemas de rotación de cultivos y en los sistemas de producción donde se busca reducir el uso de fertilizantes. Nedumaran (2015) indica que debido a las funciones que tienen los cultivos de leguminosas de grano en los sistemas agrícolas y la seguridad nutricional, la investigación sobre estos cultivos tendrá un impacto significativo en la seguridad nutricional y la fertilidad del suelo, especialmente en los países en desarrollo. Por tanto, el cultivo de leguminosas en México debiera promoverse, como parte de una estrategia de seguridad alimentaria y sustentabilidad agrícola.

A pesar de que las leguminosas constituyen una de las principales fuentes de alimentos, es común que no se les valore como debiera, debido a que existe en general desconocimiento de sus características. Martínez (1991) señala que en México hay varios géneros de leguminosas que se deben estudiar e impulsar su utilización como son *Phaseolus, Centrosema, Desmodium, Acacia y Lupinus.*

Las cualidades del lupino como fuente de proteína, mejorador del suelo (50 -150 kg de nitrógeno por ha) y su adaptabilidad a distintos tipos de suelo y clima generan expectativas de este cultivo, para la agricultura. Esta leguminosa crece y se desarrolla bien en terrenos poco fértiles (Jambrina y Crespo, 1985).

El cultivo de lupino en sus diferentes especies puede ser una fuente potencial de proteína en la producción animal, desde que se disponen de variedades libres de alcaloides y adaptadas a diferentes condiciones agroecológicas, con aceptable productividad. Las semillas de lupinos contienen más proteína que la mayor parte de las leguminosas, con excepción de la soya. El lupino es la segunda leguminosa productora de biomasa de proteína por hectárea entre 11 leguminosas de grano consideradas por la FAO (Jambrina, 1995).

El género *Lupinus* pertenece a la familia de las leguminosas. Cuatro especies son de importancia agrícola: *L. angustifolius, L. albus* y *L. luteus,* que tienen su origen alrededor del Mediterráneo y *L. mutabilis* una especie originaria de América (Mera, 2016), siendo cultivadas por su rendimiento de grano y utilización como forraje o abono verde (Nadal *et al*., 2004). Según Konieczka y Smulikowska (2017) *L. angustifolius* es actualmente la especie más difundida debido a su mayor resistencia a la antracnosis. La semilla de *L. angustifolius* es uno de los principales componentes en dietas de animales en países como Australia, Japón y Korea (Petterson, 2000).

1.1.1. Domesticación

Van de Noort (2017) resume la historia de la domesticación del lupino de la siguiente manera: el lupino (*Lupinus* spp.) se cultivó en la antigua Grecia y Egipto antes de 2000 aC, para producir granos para consumo humano y animal, cosméticos y medicinas. Alrededor de

1000 a 800 aC, *Lupinus albus* fue utilizado como abono verde en la antigua Roma y, posteriormente, en otros países mediterráneos. Dos siglos más tarde (700 a 600 aC), el lupino andino de color perla (*Lupinus mutabilis*) se domesticó en el continente americano. En la década de 1860, *Lupinus luteus* y *Lupinus angustifolius* se utilizaron para la producción de abono verde en los países Bálticos y más tarde en Alemania. Los métodos para seleccionar mutantes de lupino de bajo contenido de alcaloides se desarrollaron en Alemania a fines de la década de 1920. Más tarde, en los años 1930-1970 se desarrollaron las variedades de lupino dulce (bajo contenido de alcaloides), y luego las de semillas más permeables de *L. luteus*, *L. albus*, *L. angustifolius* y *L. mutabilis* en Alemania, Suecia y Rusia. En los años 80 y 90, se cultivaron diversas variedades de lupino en Australia y Rusia.

1.1.2. Producción de lupino a nivel mundial

El principal productor de lupino en el mundo es Australia, en 2016 produjo aproximadamente 822.9 miles de toneladas. Otros países productores de lupino incluyen a Polonia, Rusia, Marruecos, Alemania, Ucrania y Chile. De 2010 a 2016, Australia, Ucrania y Chile disminuyeron su producción; mientras que Polonia, Rusia, Marruecos y Alemania la Incrementaron (Figura 1.1).

La producción mundial de Lupino en 2016 fue de 1,284.8 miles de toneladas, la cual se ha mantenido más o menos constante en los últimos años, con excepción del año 2013 donde disminuyó considerablemente (Figura 1.2).

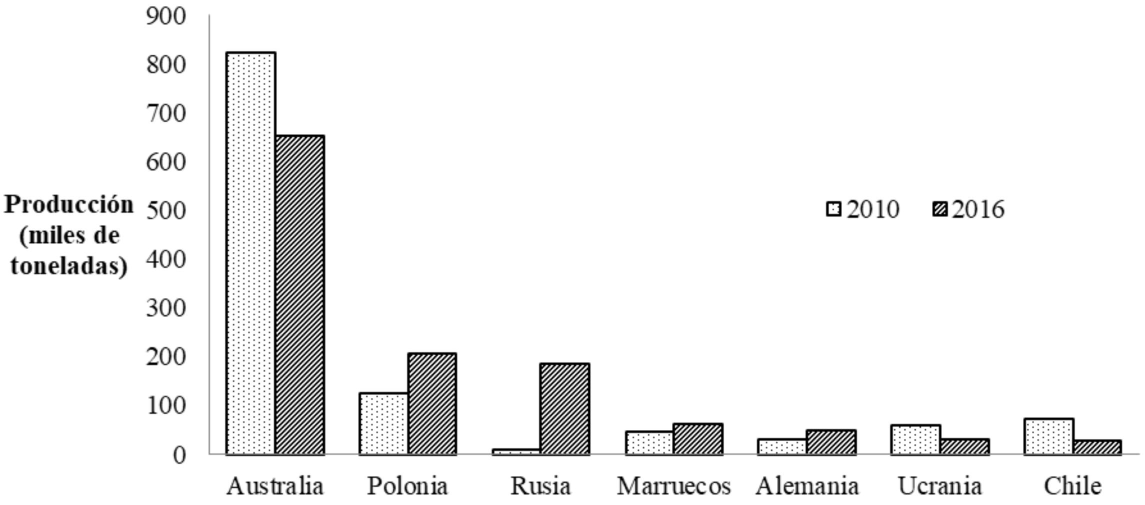

Figura 1.1. Producción anual de lupino de los principales paises productores (FAOSTAT, 2018).

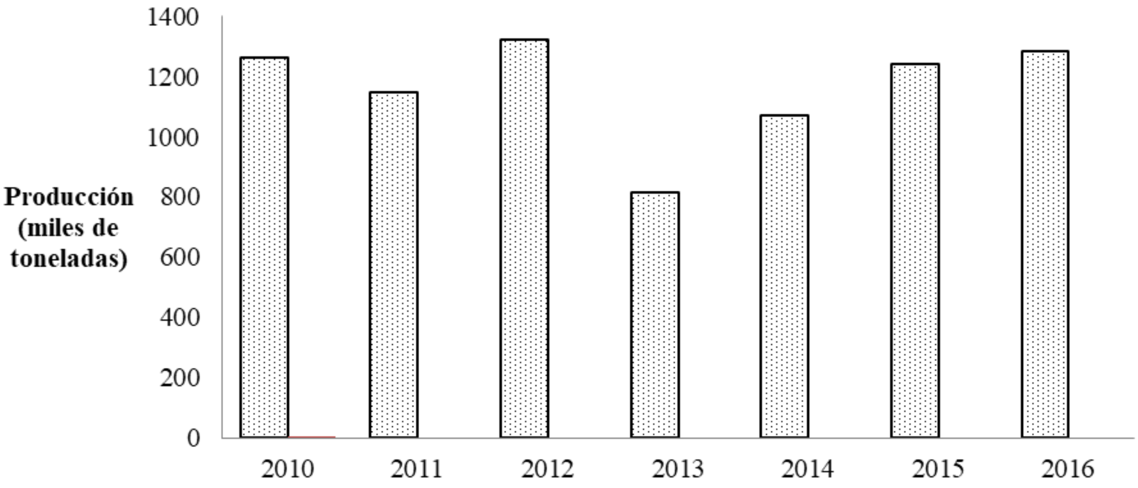

Figura 1.2. Producción mundial de lupino (FAOSTAT, 2018).

1.1.3. Requerimientos edafoclimáticos

Los lupinos pueden cultivarse en un amplio intervalo de suelos. Están adaptados a suelos arenosos, pero no se comportan bien en suelos de textura fina con mal drenaje, ya que no toleran el anegamiento (Mera, 2016). Estas plantas pueden crecer en suelos pobres, a pH bajos, menor de 6.8 para *L. angustifolius* y menos de 6 para *L. luteus* (Van de Noort, 2017). Esta característica es compartida por los rizobios que nodulan al lupino, los cuales sobreviven bien con pH de 5 a 6 y persisten por largo tiempo (Mera, 2016).

Los lupinos son resistentes al frío en sus primeras etapas de desarrollo; *L. angustifolius* es más resistente al frío (-8°C a -10°C) que *L. albus* (Van de Noort (2017).

En México la adaptación y la productividad del cultivo de lupino ha sido poco estudiado, recientemente Lara-Rivera et al. (2017) y Natera et al. (2017) presentaron resultados que sugieren que el clima y las condiciones del suelo en Zapopan, Jalisco, México, son compatibles para el cultivo de *L. angustifolius* bajo riego. Esta zona se caracteriza por un clima templado, húmedo, con lluvias de verano. Las precipitaciones varían de 700 a 1400 mm por año, con una temperatura promedio anual de 12.0 a 18.0 °C, con heladas (García, 1988). El tipo de suelo es Regosol, con una alta concentración de arena (51.8%), una baja concentración de materia orgánica (1.72%) y pH ácido (5.04; Lara-Rivera et al., 2017).

1.1.4. Practicas agronómicas

Mera (2016) indica que los lupinos se pueden sembrar con sembradora para cereales y que deben ser sembrados superficialmente (2 a 3 cm de profundidad) debido a su germinación epigea. Para *L. angustifolius* la dosis de siembra recomendable es de 80-95 kg de semilla / ha, para tener una densidad de 45 plantas / m^2. La distancia entre surcos según el mismo autor puede ser de 34 cm. También indica que el lupino está adaptado a terrenos poco fértiles y rara vez requiere fertilización.

Las condiciones del cultivo de *L. angustifolius*, durante el ciclo otoño-invierno, en Zapopan, Jalisco, señaladas por Lara-Rivera et al. (2017) consisten en una preparación convencional del terreno con un arado de vertedera para el barbecho y paso de rastra de discos. Posteriormente se realizó la siembra manualmente en surcos distanciados a 80 cm y 10 cm entre plantas (con una densidad aproximada de 12-13 plantas / m^2). Después de la siembra, se aplica riego por goteo a capacidad del campo; con una frecuencia de cada 15 días y el cual se suspende 10 días antes de la cosecha. Debido a la presencia limitada de malezas, éstas se eliminan manualmente durante el cultivo. Las semillas no fueron inoculadas y no se utilizaron fertilizantes, debido a que se considera que el lupino prospera bien en suelos de baja fertilidad. La aplicación de pesticidas no fue necesaria para el crecimiento y desarrollo del cultivo debido a la baja incidencia de plagas y enfermedades.

Zamora et. al. (2017) evaluaron seis cultivares de *Lupinus angustifolius* durante dos años en suelos ácidos en Zapopan, Jalisco México, y reportaron que el rendimiento de grano varió de 2850 a 5016 kg por ha. Estos autores sugieren que el cultivo de lupino en México puede alcanzar rendimientos comparables a los alcanzados en áreas donde el lupino es un cultivo tradicional.

1.2. Composición química y energía metabolizable de la semilla de lupino

La semilla de lupino (*L. angustifolius*) contiene aproximadamente 34% de proteína (van de Noort, 2017) similar al frijol soya, pero menor que la pasta. No obstante, este nivel permite formular dietas para aves o cerdos con semilla de lupino como única fuente de proteína si se suplementan aminoácidos sintéticos. Las semillas descascarilladas de lupino pueden tener más de 40% de proteína y mayores concentraciones de aminoácidos y valores energéticos que la semilla entera (Nalle et al. 2010). Aunque el lupino no se considera como una oleaginosa, tiene una cantidad considerable de aceite en la semilla (5 a 6%), la cual puede ser importante como aporte de energía y fuente de ácidos grasos esenciales. Los dos principales ácidos grasos encontrados en el aceite de las semillas de *L. angustifolius* son el ácido oleico y el ácido linoleico (Hansen y Czochanska, 1974). La semilla de lupino, desde el punto de vista de su composición química, es una alternativa importante para sustituir a la pasta de soya en la alimentación animal.

La composición nutricional de los lupinos está bien documentada. Sin embargo, la variabilidad nutricional entre variedades (Sipsas y Glencross, 2005) son limitaciones importantes en el uso de esta leguminosa en dietas para aves. En el Cuadro 1.1, se presenta el promedio y la variación en la composición nutricional de diferentes variedades, con base en reportes recientes.

1.2.1. Proteína y aminoácidos

El contenido de proteína de semillas enteras de *L. angustifolius* presenta variación en función de la variedad desde 26.5% reportado por Nalle et al. (2011) para la variedad Tanjil hasta 38.6% reportado por Hejdysz et al. (2018) para la variedad Boruta. Esta diferencia entre variedades, de más de 12 unidades porcentuales, es de consideración al momento de formular dietas y evidencia la necesidad de caracterizar nutricionalmente y con precisión la variedad.

Las proteínas de la semilla de lupino son proteínas de reserva; la mayor cantidad (85%) corresponde a globulinas y una pequeña proporción (15%) de albuminas (Petterson, 1998). Según Glencross (2001), las proteínas de la semilla de lupino tienen un contenido alto de arginina, lisina, leucina y fenilalanina comparado con el del frijol soya. Sin embargo, una notable diferencia es su deficiencia comparativa de metionina y cistina.

Cuadro 1.1. Composición química (% base seca) de semilla entera de *Lupinus angustifolius*.

Nutriente	**Promedio**	**Desviación Estándar**	**Mínimo**	**Máximo**	**Fuente**
Materia seca	89.02	1.03	88.00	91.00	2, 3, 4, 5
Proteína cruda	31.41	3.85	26.50	38.58	1, 2, 3, 4, 5
Extracto etéreo	5.62	0.75	4.80	7.33	1, 2, 3, 5
Fibra cruda	15.55	1.33	13.80	17.89	1, 3, 5
Cenizas	3.55	0.36	3.00	4.01	2, 3, 4, 5
FDN	24.68	1.10	23.24	25.92	1, 4
FDA	20.60	0.83	19.48	21.43	1, 4
Calcio	0.38	0.01	0.37	0.39	1
Fósforo	0.58	0.03	0.55	0.60	1

Referencias: 1. Vecereck et al., 2008; 2. Nalle et al., 2011; 3. Smulikowska et al., 2014; 4. Hejdysz et al., 2018; 5. Konieczka y Smulikowska, 2018.

La concentración de aminoácidos en semillas enteras de algunas variedades de *Lupinus angustifolius* se presenta en el Cuadro 1.2. Su perfil de aminoácidos es similar al de otras proteínas de leguminosas por su bajo contenido de aminoácidos azufrados y triptófano. Sin embargo, las semillas de lupino son consideradas como una excelente fuente de arginina.

La digestibilidad ileal aparente de los aminoácidos de semillas enteras de algunas variedades lupino, reportada por Nalle et al. (2011) se muestra en el Cuadro 1.3.

En general, se encontró que los coeficientes de digestibilidad promedio de los aminoácidos esenciales y no esenciales están por encima de 0.83. Entre los aminoácidos indispensables, la arginina tuvo el coeficiente de digestibilidad más alto (0.92 – 0.95), mientras que la metionina presentó el más bajo (0.74 – 0.83).

Cuadro 1.2. Concentración de aminoácidos (% base seca) de semilla entera de tres variedades de *Lupinus angustifolius*.

Aminoácidos	**Wallan**	**Tanjil**	**Borre**
Esenciales			
Arginina	3.180	2.60	3.24
Histidina	0.851	0.799	0.899
Isoleucina	1.100	0.988	1.170
Leucina	1.920	1.750	2.130
Lisina	1.540	1.490	1.590
Metionina	0.255	0.253	0.244
Fenilalanina	1.040	0.979	1.110
Treonina	1.250	1.200	1.340
Valina	1.150	1.030	1.200
No esenciales			
Alanina	1.110	1.050	1.150
Acido aspártico	2.720	2.530	2.970
Cistina	0.548	0.490	0.524
Glicina	1.180	1.030	1.270
Acido glutámico	5.930	4.700	6.530
Prolina	0.988	0.856	1.110
Serina	1.090	0.993	1.270
Tirosina	0.966	0.886	1.070

Referencia: Nalle et al., 2011

Cuadro 1.3. Coeficientes de digestibilidad ileal aparente de aminoácidos de semilla entera en tres variedades de *Lupinus angustifolius*, con pollos de engorda.

Aminoácidos	**Wallan**	**Tanjil**	**Borre**
Esenciales			
Arginina	0.95	0.94	0.92
Histidina	0.80	0.77	0.80
Isoleucina	0.86	0.84	0.85
Leucina	0.91	0.86	0.85
Lisina	0.86	0.87	0.87
Metionina	0.74	0.80	0.83
Fenilalanina	0.92	0.90	0.84
Treonina	0.82	0.80	0.84
Valina	0.84	0.83	0.82
Promedio	0.85	0.84	0.85
No esenciales			
Alanina	0.85	0.83	0.81
Acido aspártico	0.83	0.84	0.85
Cistina	0.84	0.83	0.82
Glicina	0.83	0.81	0.83
Acido glutámico	0.92	0.91	0.90
Prolina	0.87	0.77	0.81
Serina	0.86	0.77	0.83
Tirosina	0.86	0.83	0.82
Promedio	0.86	0.82	0.83

Referencia: Nalle et al., 2011

1.2.2. Lípidos y ácidos grasos

Chiofalo et al. (2012) reportaron valores de aceite de 3.28 a 4.66% en semillas de variedades de *Lupinus angustifolius*, con contenidos de 21.03 a 26.66% de ácidos grasos saturados, de 29.49 a 38.69% de ácidos grasos monoinsaturados, de 28.70 a 43.19% de ácidos grasos poliinsaturados n-6 y de 5.90 a 7.66% de ácidos grasos poliinsaturados n-3 (Cuadro 1.4).

Cuadro 1.4. Principales ácidos grasos del aceite de semilla de variedades de *Lupinus angustifolius*.

Ácido graso[1]	**Chiofalo et al. (2012)**[2]	**Hansen y Czochanska (1974)**[3]	
	Semillas enteras	**Semillas enteras**	**Cotiledones**
Contenido de aceite (%)	3.28 – 4.63	8.6	10.0
Palmítico (C16:0)	10.83 – 12.14	7.6	8.0
Esteárico (C18:0)	5.10 – 7.74	4.6	5.2
Behenico (C22:0)	2.90 – 4.33	1.7	1.9
Oleico (C18:1n9)	28.13 – 36.89	31.2	33.6
Linoleico (C18:2n6)	28.62 – 43.15	48.3	44.8
Alfa-linolénico (18:3n3)	5.90 – 7.66	5.4	5.0

[1] La concentración de ácidos grasos se expresó por el total de ésteres metílicos de ácidos grasos identificados.

[2] Rango de promedios encontrados para tres variedades (“Wonga”, “Jindalee” y “Sonet”).

[3] Medias obtenidas para la variedad “Uniwhite”

Entre los ácidos grasos saturados destacan el ácido palmítico (C16:0), el ácido esteárico (C18:0) y el ácido behenico (C22:0), en las semillas de *L. angustifolius*; mientras que en los monoinsaturados destaca el ácido oleico (C18:1n9) y el los polinsaturados el ácido linoleico (C18:2n6) y el ácido alfa-linolénico (18:3n3). Estos dos últimos considerados como ácidos grasos esenciales, lo cual es importante desde el punto de vista nutricional (Chiofalo et al., 2012).

Los dos principales ácidos grasos encontrados en el aceite de las semillas de *L. angustifolius* son el ácido oleico (C18:1n9) y el ácido linoleico (C18:2n6), en ocasiones constituyendo hasta un 79.5 y 78.4% de total de lípidos en semillas enteras y cotiledones, respectivamente (Hansen y Czochanska, 1974).

1.2.3. Valores de energía metabolizable aparente de semilla de lupino para pollos de engorda

Nalle et al. (2011) reportaron que los valores de energía metabolizable aparente (EMA) y EMA corregida por nitrógeno (EMAn) de semillas enteras de *Lupinus angustifolius* difieren entre variedades (Cuadro 1.5). Smulikowska y Rutkowski (2005) señalan que la EMA de semilla de lupino es de 7.22 MJ / kg para pollos de engorda. Por otro lado, Hughes y Choct (1999) indican que los valores de AME de cotiledones de semilla de lupino varían de 8.0 a 12.3 MJ / kg de MS para *L. angustifolius* cv. Gungurru y 6.5 a 10.5 MJ / kg MS para *L. angustifolius* cv Danja.

Cuadro 1.5. Valores de energía metabolizable aparente (EMA) y EMA corregida por nitrógeno (EMAn) de semilla entera de variedades *Lupinus angustifolius*.

Variedad	**EMA (MJ / kg)**	**EMAn (MJ / kg)**
Wallan	6.38	5.35
Tanjil	6.73	6.18
Borre	7.12	5.52

Referencia: Nalle et al., 2011.

1.3. Factores anti-nutricionales de la semilla de lupino

Los principales factores antinutricionales de los lupinos son los alcaloides, la fracción soluble de los polisacáridos no amiláceos, los oligosacáridos de la familia de la rafinosa y el fósforo en forma de fitatos (Nalle et al., 2010; Nalle et al., 2011; Kaczmarek et al., 2014). Sus concentraciones varían entre especies de lupinos, cultivares y condiciones ambientales (Castell et al., 1996). En el Cuadro 1.6 se muestran las concentraciones promedio de los factores antinutricionales en las principales especies de lupino.

Cuadro 1.6. Factores antinutricionales en las principales especies de lupino (promedios de las cifras mundiales).

	L. albus	***L. angustifolius***	***L. Luteus***
Alcaloides totales (mg / kg)	< 200	< 200	200-500
Oligosacáridos (%)	7.5	5.2	12.3
Saponinas (mg / kg)	< 1	570	55
Taninos condensados (%)	0.01	< 0.01	0.02
Lectinas (actividad)	Traza	Traza	Traza
Inhibidores de tripsina (mg / g)	0.13	0.14	0.29
Fitatos (%)	0.79	0.58	0.96

Referencia: Petterson (2016)

1.3.1. Alcaloides

Históricamente, los alcaloides fueron el principal factor que limitó el uso de lupinos en la nutrición de animales no rumiantes. Los lupinos silvestres son amargos y tóxicos porque producen alcaloides quinolizidínicos como medio de defensa química. El contenido total de alcaloides en los lupinos silvestres (amargos) generalmente está en el intervalo de 10 a 45 g por kg (Trugo et al., 2003). Se han producido muertes humanas asociadas con el consumo de grano de lupino silvestre (Petterson, 2016). Mediante fitomejoramiento, se mejoró el valor nutricional de los lupinos y el contenido de alcaloides de los cultivares nuevos oscila entre 0.08 y 0.12 g / kg, por lo que se pueden utilizar tanto para consumo humano como animal (Jezierny et al., 2010).

En un estudio realizado por Olkowski et al. (2001) donde se avaluó el uso de semilla lupino (cv. Troll) entero (400 g / kg), sin cáscara (350 g / kg) y sometida a autoclave (400 g / kg) en dietas para pollos de engorda, observaron que las aves alimentadas con dietas basadas en lupino tuvieron menor crecimiento y consumo de alimento en comparación con pollos alimentados con la dieta de testigo a base de pasta de soya. Se observaron signos agudos de intoxicación (debilidad de las patas, falta de coordinación y tortícolis) en algunos pollos que recibieron la dieta con lupino entero durante la primera semana de experimentación. Durante las semanas dos y tres, algunas aves que recibieron la dieta con lupino entero mostraron signos de parálisis muscular y deformación esquelética. Concluyeron que los altos niveles de alcaloides en algunas variedades de lupinos “dulces” podrían causar efectos perjudiciales significativos en pollos de engorda.

1.3.2. Oligosacáridos

Los oligosacáridos del lupino son α-galactósidos como rafinosa, estaquiosa y verbascosa (Petterson, 2016) Estos oligosacáridos varían de 7 a 15% en semillas crudas (Van de Noort, 2017).

Los oligosacáridos de la familia de la rafinosa se consideran factores antinutricionales en los alimentos para humanos. El consumo de altos niveles de estos oligosacáridos reduce la absorción de glucosa y metionina (Martínez-Villaluenga et al., 2008). Los oligosacáridos de este tipo no se digieren ni se absorben en el tracto gastrointestinal superior de los no rumiantes; pasan al intestino grueso y se fermentan anaeróbicamente por la microflora intestinal en ácidos grasos de cadena corta y gases (dióxido de carbono, metano e hidrógeno) que pueden provocar flatulencia y malestar abdominal (Martínez-Villaluenga et al., 2008; Petterson, 2016)

Kocher et al., (1999), indican que los oligosacáridos de los cotiledones de *L. angustifolius* contribuyen al contenido de energía metabolizable en la dieta de pollos de engorda. Ellos concluyen que los oligosacáridos de esta especie de lupino no representan un compuesto estrictamente antinutritivo en pollos como se pensaba.

1.3.3. Saponinas

Las saponinas son glucósidos vegetales en los que la parte no azúcar es un esteroide o un compuesto triterpenoide. Su sabor amargo es un inconveniente para su uso, pueden alterar la función del epitelio intestinal haciendo que la mucosa sea más permeable, lo que facilita la absorción de materiales a los que el intestino normalmente no sería permeable (Petterson, 2016). Las saponinas no están presentes en *L. albus*, pero en las especies de *L. luteus, L. mutabilis* y *L. angustifolius* la concentración puede variar de 57 a 470 mg / kg (Trugo et al., 2003) o hasta 730 mg / kg (Petterson, 2016); sin embargo, estas cifras son todavía bajas en comparación con el frijol soya crudo, que presentan valores de 2000 a 5000 mg / kg (Trugo et al. 2003).

Las soponinas son tóxicas para los peces y retrasan el crecimiento en el ganado y en animales de laboratorio (Trugo et al., 2003)

1.3.4. Taninos

Los taninos son sustancias polifenólicas complejas, con la propiedad de precipitan a las proteínas en medio acuoso. Interactúan con una o más moléculas de proteína formando grandes complejos que son insolubles en agua. Esta propiedad hace que los taninos sean indeseables ya que harán que la proteína dietética no sea digerible. Las semillas de lupino presentan valores relativamente bajos de taninos de 0.2 a 0.5%. No se ha encontrado ninguna correlación entre el contenido de taninos y el sabor amargo de algunas semillas de lupino, y el impacto de los taninos de la semilla de lupino en la dieta aún no se ha evidenciado en humanos ni en animales (Trugo et al., 2003).

El contenido de taninos en *L. angustifolius* se ha considerado suficientemente bajo para su uso en dietas de cerdos sin ningún problema (Trugo et al., 2003). Sin embargo, los taninos pueden aumentar con la fertilización (Lampert-Szczapa, 2003).

1.3.5. Lectinas e inhibidores de tripsina

Se ha encontrado que la actividad del inhibidor de la tripsina es insignificante (< 1 unidades de inhibidor de tripsina (TIU) / mg) en algunos cultivares de *L. angustifolius* y *L. albus* (Nalle et al., 2011; Nalle et al., 2012). La actividad de la lectina es prácticamente inexistente en todas las especies de lupinos (Petterson. 2016). Los inhibidores de tripsina se destruyen con calor (Leeson y Summers, 2015). A diferencia de la mayoría de los granos de leguminosas, los lupinos no necesitan procesamiento térmico y pueden ser incorporados a la dieta de los animales en forma cruda (Nalle et al., 2012).

Los inhibidores de tripsina impiden la digestión de las proteínas y su presencia se caracteriza por una hipertrofia del páncreas. Además de la disminución de la ganancia del peso y la producción de huevo, la presencia de inhibidores es diagnosticada por un incremento de 50-100% en el tamaño del páncreas (Leeson y Summers, 2015). Nalle et al. (2012) observaron que pollos de engorda en iniciación alimentados con dietas con 20% de semilla entera de *L. albus* (cv. Kiev) tenían un peso relativo del páncreas más bajo ($P < 0.05$) que en las aves alimentadas con una dieta de maíz-soya. Esto posiblemente está relacionado con las cantidades insignificantes de inhibidores de tripsina en la semilla de lupino.

1.3.6. Fitatos

Los fitatos se consideran un factor antinutricional porque están implicados con una inadecuada absorción de minerales. Se encuentra en las semillas de lupino aproximadamente en el mismo rango de concentración que en otras leguminosas, pero por lo general es más bajo que en la soya. La cantidad promedio encontrada en la semilla de lupino es de alrededor de 0.8 g / 100 g (Trugo et al., 2003).

Los fitatos causan una reducción significativa en el comportamiento productivo de los pollos. Sus principales efectos perjudiciales incluyen la reducción de la biodisponibilidad de los minerales, la disminución de la actividad de las enzimas digestivas y la menor disponibilidad de otros componentes de la dieta (Ravindran et al., 2000).

En general aproximadamente 33% del fósforo que contienen los ingredientes de origen vegetal usados en la alimentación de no rumiantes está presente como fitatos. Los no rumiantes no tienen fitasas, por lo que la disponibilidad del fósforo es baja. La formación de fitatos hace al Ca y P insolubles y también forma complejos con varios cationes, pero el Zn y el Cu tienen mayor afinidad para unirse. Esta unión hace que estos minerales no se puedan absorber en el intestino (Ravindran et al., 2000).

1.3.7. Polisacáridos no amiláceos

Los polisacáridos no amiláceos (PNA) de los lupinos son un factor antinutricional importante en la alimentación de los animales no rumiantes porque tienen un efecto perjudicial en la fisiología gastrointestinal y disminuyen la utilización de nutrientes y energía, lo que puede afectar los parámetros productivos (Steenfeldt et al., 2003).

Los PNA de los lupinos consisten principalmente en celulosa, arabinoxilanos y polisacáridos pécticos, estos últimos son estructuras complejas altamente ramificadas que consisten en cadenas principales de ramnogalacturonano con largas cadenas laterales de α-arabinanos y β-galactanos (Cheetham et al., 1993).

La concentración de PNA, es casi dos veces más alto en los lupinos que en otras fuentes de proteína vegetal (Bach Knudsen, 1997), restringen el uso su semilla en las dietas para aves, ya que los PNA no se digieren en el sistema digestivo superior de las aves debido a la ausencia de enzimas endógenas específicas (Annison et al., 1996; Smits y Annison, 1996). Se han propuesto dos alternativas para explicar el efecto antinutricional de los PNA en las dietas para pollos de engorda; la encapsulación en la que la capa de PNA, impide el acceso de las enzimas digestivas al almidón, la grasa y la proteína, y a que la presencia de PNA en el intestino aumenta la viscosidad del quimo (Caprita et al., 2010).

Los principales efectos antinutricionales en aves y cerdos están dados por la parte soluble de PNA, ya que puede disminuir la digestibilidad de los nutrientes al cambiar las condiciones en el intestino al aumentar la viscosidad del quimo (Choct, 1997). A medida que aumenta la viscosidad, disminuye la difusión de enzimas, sustratos y productos (Petterson y Aman, 1989), lo que dificulta la mezcla de nutrientes con enzimas pancreáticas y ácidos biliares. Además, una dieta alta en PNA puede causar una disminución en la longitud, el ancho y la superficie de las vellosidades, lo que tiene un efecto negativo en la absorción de nutrientes (Mathlouthi et al., 2002). De esta manera, los PNA pueden reducir la digestión y la absorción de nutrientes debido al efecto físico-químico en el sistema digestivo intestinal (Caprita et al., 2010).

1.4. Mejoramiento del valor nutrimental de la semilla de lupino

1.4.1. Descascarado

Los efectos favorables del descascarado de la semilla de lupino se evidenciaron en el estudio de Vecereck et al. (2008), quienes reportaron (en semillas de diversas variedades de *Lupinus albus*, *Lupinus angustifolius* y *Lupinus luteus*) un incremento en la concentración de proteína y aceite y una disminución de la fibra. La semilla descascarada contenía en promedio 46.7% de proteína comparada con la semilla entera con sólo 37.6%; el contenido de fibra cruda fue de 14.8% en la semilla entera contra sólo 2.8% en la semilla descascarada.

Brenes et al. (2003) por su parte informaron que el descascarado incrementa el contenido de proteína en aproximadamente 20% y reduce el contenido de fibra en aproximadamente 70% en las semillas de lupino. Así mismo, Nalle et al. (2010) informaron que el descascarado mejoró el valor de EMA (6.29 MJ / kg frente a 8.20 MJ / kg) y EMAn

(5.82 MJ / kg frente a 7.39 MJ / kg) de la semilla de *L. angustifolius*. Sin embargo, señalan que los coeficientes de digestibilidad ileal aparente de los aminoácidos no se vieron afectados por la remoción de la cáscara.

1.4.2. Uso de enzimas exógenas

La suplementación de enzimas a las raciones de aves tiene un efecto positivo en la digestibilidad de los alimentos y conduce a una mejor productividad y rendimiento. Además, la suplementación de enzimas comerciales puede aumentar el valor nutritivo de los ingredientes de los alimentos y las dietas, así como permitir una mayor flexibilidad en la formulación de la dieta. También tiene un efecto potencial en la mitigación de la contaminación ambiental al reducir la excreción de algunos elementos como el nitrógeno y el fósforo en la excreta de las aves (Alagawany et al., 2018)

1.5. Uso de la semilla de lupino en la alimentación animal

Los resultados de la inclusión de semillas de lupino en la dieta de animales no rumiantes han sido variables. A continuación, se indican resultados positivos en la utilización de lupino en alimentación de animales. Parece ser que el éxito de la inclusión de lupino está relacionado con el hecho de que se balanceen adecuadamente las dietas, se incluyan aminoácidos sintéticos para cubrir las necesidades, se cuiden los niveles de alcaloides y / o se incluyan enzimas que degraden los polisacáridos no amiláceos.

Lee et al. (2016) evaluaron la inclusión semilla entera y descascarada *L. angustifolius* y la suplementación de enzimas en la dieta de las **gallinas ponedoras** durante el crecimiento y 12 semanas de la fase de postura. Ellos observaron que la inclusión de 15% de semilla lupino en la dieta no produjo efectos adversos en la producción o la salud de la gallina; por lo que concluyeron que podría usarse como parte de una ración balanceada con inclusión de enzimas degradadoras de PNA.

Recientemente, Kasprowicz-Potocka et al. (2016) evaluaron el valor nutricional de cuatro variedades de *L. angustifolius* en **cerdos de engorda** y concluyeron que la sustitución total de pasta de soya por semilla de lupino de la variedad Sonate no tiene efectos negativos en el comportamiento productivo de cerdos en crecimiento y finalización. Estos resultados concuerdan con Kim et al. (2007) quienes realizaron una revisión acerca del uso de lupinos como fuente de proteína en las dietas de cerdos y concluyeron las especies *L. angustifolius* y *L. luteus* pueden reemplazar completamente a la pasta de soya en las dietas para cerdos en las etapas de crecimiento y finalización sin afectar el comportamiento productivo. Sin embargo, para ser utilizadas eficientemente por los cerdos, las dietas que contienen lupinos requieren una formulación que responda a avrios aspectos como: la menor proporción de energía digestible con mayores contenidos de carbohidratos fermentables; suplementación de aminoácidos sintéticos (lisina, metionina, treonina y triptófano), ya que son deficientes o se pierden a través del flujo endógeno de aminoácidos. Adicionalmente, según estos autores, la suplementación de mezclas enzimáticas específicas, es un enfoque prospectivo para mejorar el valor nutritivo de los lupinos.

Volek y Marounek (2009) evidenciaron que la semilla de *Lupinus albus* (cv. Amiga) es una fuente adecuada de proteína para los **conejos** de engorda, que pueden reemplazar completamente a las fuentes de proteína utilizadas tradicionalmente como la pasta de soya o harina de girasol.

Petterson (1998) revisó los registros de producción de una estación de investigación que estudia el comportamiento productivo de los cerdos alimentados con semillas de lupino. Ocho generaciones de cerdos y un total de más de 1100 camadas habían sido alimentadas con dietas de crianza o finalización con 10-40% de semilla de *L. angustifolius* y dietas de cerdas con 10-30%. Los niveles de alcaloides en las dietas se estimaron en un rango de 10-80 mg / kg. No se encontraron indicios de efectos teratológicos o lesiones significativas en cerdas sacrificadas. Los datos de producción fueron comparables a los datos de producción de la industria porcina que no usaron dietas de lupino.

1.5.1. Utilización de la semilla de lupino en dietas para pollos de engorda

Mierlita y Popovici (2013) evaluaron el comportamiento productivo de pollos de engorda en relación a la sustitución parcial de pasta de soya por semilla lupino en la dieta. La sustitución de hasta 30% de pasta de soya en la etapa inicial (primeras tres semanas de edad) y de hasta 60% en las etapas subsecuentes (de la cuarta a la sexta semana) por harina de semilla de lupino no afectó el comportamiento productivo. Porcentajes más altos de sustitución de la pasta de soya por harina de lupino (40% y 80%, en las fases correspondientes) disminuyeron el comportamiento productivo. En esta investigación además se evidenció la disminución de los costos de la dieta al incluir lupino y, por tanto, el incremento de los beneficios económicos con estas dietas. Sin embargo, se concluyó que la semilla lupino entera no se pueden utilizar como única fuente de proteína debido a su contenido alto de polisacáridos no amiláceos, lo cual aumenta la viscosidad en el contenido intestinal, que a su vez afecta el consumo y la utilización del alimento.

Nalle, et al., (2011) estudiaron el efecto de la alimentación con dietas con 20% de semilla de variedades de *L. angustifolius* en el comportamiento productivo y desarrollo del sistema digestivo de pollos de engorda en la fase de iniciación. Los resultados de este estudio indican que, cuando las dietas están balanceadas en energía metabolizable y aminoácidos digestibles, se puede incluir 20% de semilla entera de lupino como sustituto parcial de la pasta de soya en dietas de iniciación de pollos de engorda, sin efectos adversos en el comportamiento productivo.

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CAPÍTULO II

SOYBEAN MEAL SUBSTITUTION BY DEHULLED LUPINE (*Lupinus angustifolius)* WITH ENZYMES IN BROILER DIETS

2.1. Abstract

Objective: Evaluate the effects of 1) dehulling of lupine seed on chemical composition and apparent metabolizable energy (AME) and 2) soybean meal substitution by dehulled lupine seed in broiler diets with enzymes on productive performance, size of digestive organs and welfare-related variables.

Methods: Experiment 1. Chemical composition and AME were determined in whole and dehulled lupine seed. Experiment 2. Two hundred eighty-eight one-day-old male Ross 308 broilers were used. The experimental diets were maize-soybean meal (MS), MS with enzymes (MSE) and maize-dehulled lupine seed with enzymes (MLE). Diets were assigned to the experimental units under a completely randomized design (eight replicates per diet). The BW gain, feed intake, feed conversion, digestive organ weights, gait score, latency to lie down and *valgus/varus* angulation were evaluated.

Results: The dehulling process increased protein (25.0 to 31.1%), AME (5.9 to 8.8 MJ/kg) and amino acid contents. The BW gain of broilers fed the MLE diet was similar ($P > 0.05$) to that of those fed the MS diet, but lower than that of those fed the MSE diet. Feed intake of broilers fed the MLE diet was higher ($P < 0.05$) than that of those fed the MS diet and similar ($P > 0.05$) to those fed the MSE diet. Feed conversion of broilers fed the MLE diet was 8.0 and 8.7% higher ($P < 0.05$) than that of those fed the MS and MSE diets, respectively. Broilers fed the MLE diet had the highest ($P < 0.05$) relative proventriculus and gizzard weights, but had poor welfare-related variables.

Conclusion: It is possible to substitute soybean meal by dehulled lupine seed with enzymes in broiler diets, obtaining similar BW gains in broilers fed the MLE and MS diets; however, a higher feed intake is required. Additionally, the MLE diet reduced welfare-related variables.

Keywords: Apparent Metabolizable Energy; Enzymes; Welfare-Related Variables; Broiler Performance; Digestive Organ Size.

2.2. Introduction

Dependence on soybean meal as a protein source in animal feed poses environmental and economic problems. The price of this ingredient has significantly increased in recent years due to the growing demand for protein ingredients (Lee et al., 2016). Consequently, there is a need to find and assess alternative sources of protein for animal feed. In this context, *Lupinus angustifolius* seed might be an alternative protein source due to its high protein and oil contents.

Study of chemical composition and apparent metabolizable energy (AME) of the ingredients is basic for assessing its use in animal feed. Protein and energy constitute around 90% of the total cost of poultry feeding (Perween et al., 2016), and the chemical composition and AME content of the ingredients determine broiler productive performance (Francesch, 2001). The chemical composition of lupine seed varies depending on the cultivar. Vecerek et al. (2008) showed that, while chemical composition of lupine seed varies among cultivars, dehulling improves seed nutritional value, increasing protein and oil contents and decreasing fiber concentration. These results are of interest for non-ruminants that cannot digest fiber. However, there are few reports about chemical composition and AME of dehulled lupine seed.

Whole lupine seed cannot be used as a single source of protein in diets for broilers due to its high level of non-starch polysaccharides (NSP), which increase viscosity of intestinal content and affect feed intake and utilization (Mierlita and Popovici, 2013). Total NSP content ranges from 43.2 to 49.6% in whole seeds of *Lupinus angustifolius* cultivars (Nalle et al. 2011). Evans et al. (1993) found 29 to 31% NSP in the cotyledons and 86 to 89% in the hull. Nalle et al. (2011) suggested that it is possible to include high levels of lupine in diets for broilers by removing the hull and/or by using enzymes to degrade NSP. The use of exogenous enzymes such as xylanases and proteases in diets for broilers to attenuate nutrient encapsulation by NSP, such as arabinoxylans, and to increase protein degradation has recently been investigated (Pasquali et al., 2017). We hypothesized that inclusion of dehulled lupine seed as the main protein source plus enzymes in broiler diets does not affect productive performance, size of digestive organs or welfare-related variables. The objectives of this study were to determine the effect of the dehulling process of lupine seed on its chemical composition and AME and to evaluate the inclusion of dehulled lupine seed as the main protein source in diets supplemented with enzymes, on productive performance, size of digestive organs and welfare-related variables in broilers.

2.3. Materials and methods

The broilers were cared for following the guidelines established by the Animal Welfare Committee of the Colegio de Postgraduados.

2.3.1. Experiment 1. Chemical composition and apparent metabolizable energy of whole and dehulled lupine seed

Lupine (*L. angustifolius* cv. Boregine) seed was produced under irrigation during the autumn-winter crop cycle (2014-2015) in Jalisco, Mexico, situated at 20° 43' N and 103° 23' W at an altitude of 1560 m.

Chemical analysis

Whole and dehulled seed were ground and sieved through a 0.5 mm mesh. Dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca) and phosphorus (P) contents were determined according to the AOAC methods (1990), three samples were analyzed for each fraction. Amino acid concentration was determined by high performance liquid chromatography (HPLC), by Evonik Industries de México, SA de CV.

Determination of apparent metabolizable energy

AME was measured in the three experimental diets: 1) basic diet of maize-soybean meal (Table 2.1), 2) 75% basic diet plus 25% whole lupine seed, and 3) 75% basic diet plus 25% dehulled lupine seed.

One-day-old male Ross 308 strain broilers (n = 100) were raised in cages and fed a starter diet (22% CP and 12.55 MJ ME/kg) up to 21 days of age and a finisher diet (20% CP and 12.55 MJ ME/kg) from 22 to 30 days of age. On day 30, sixty broilers were selected according to their body weight (1.7 ± 0.1 kg) and assigned to 30 cages (2 broilers per cage). The cages (30 × 60 × 40 cm) had a linear feeder and an automatic-cup drinker in the front. The diets were given in mash form and randomly assigned to ten replicates. Feed and water were available *ad libitum*.

AME was determined using the classical total excreta collection method. From day 38 to 42 of age, feed intake and excreta output were measured quantitatively per cage. Feathers, scales, and feed scraps were removed from the excreta. To minimize feed waste, over-filling the feeders was avoided. For AME determination a pooled sample of excreta from each cage was taken and stored at -20°C for subsequent lyophilization (Labconco, Labconco Corporation, Kansas City, Missouri USA). Dried excreta and diet samples were ground to pass through a 0.5 mm sieve and stored in sealed plastic containers for DM, gross energy (GE) and nitrogen content analysis. The GE was determined using an isoperibol bomb calorimeter (PARR 1266, Parr Instrument Company, Moline, Illinois USA). Nitrogen content was measured by the Kjeldahl method (AOAC, 1990).

Table 2.1. Ingredient composition (% as fed) and calculated analysis (% dry matter) of the basic diet used to determine apparent metabolizable energy, Experiment 1.

Ingredient	**%**
Maize	59.360
Soybean meal	35.180
Soybean oil	1.780
Dicalcium phosphate	2.170
Calcium carbonate	0.780
Sodium chloride	0.200
Sodium bicarbonate	0.230
Mineral-vitamin premix	0.300
Calculated analysis	
Metabolizable energy (MJ/kg)	12.58
Crude protein	20.55
Lysine	1.10
Methionine	0.30
Methionine + cystine	0.67
Calcium	0.84
Available phosphorus	0.49

Provided per kg of diet, vitamins: A, 12,000 UI; D3, 1,000 UI; E, 60 UI; K, 5.0 mg; B2, 8.0 mg; B12, 0.030 mg; pantothenic acid, 15 mg; niacin, 50 mg; folic acid, 1.5 mg; choline, 300 mg; biotin, 0.150 mg; thiamine, 3.0 mg. Minerals: Fe, 50.0 mg; Zn, 110 mg; Mn, 100 mg; Cu, 12.0 mg; Se, 0.3 mg; I, 1.0 mg.

Calculations

The AME values of lupine seed were calculated using the following formulas (dry basis).

AME_{diet} (MJ/kg) = [(feed intake × GE_{diet}) – (excreta output × $\text{GE}_{\text{excreta}}$)] / feed intake

$\text{AME}_{\text{lupine seed}}$ (MJ/kg) = [(AME of lupine diet) – (AME of basal diet × 0.75)] / 0.25

The nitrogen-corrected AME (AMEn) value was calculated using a factor of 34.4 kJ per g nitrogen retained.

2.3.2. Experiment 2. Productive performance, size of digestive organs and welfare-related variables in broilers fed dehulled lupine seed plus enzymes

The experiment was carried out in the poultry facilities of the Colegio de Postgraduados, Campus Montecillo, situated in Texcoco, State of Mexico, Mexico, at 19° 29' N, 98° 54' W, and an altitude of 2,247 m. The lupine seed cultivar Boregine was obtained from an irrigated field during the autumn-winter cycle (2015-2016) in Zapopan, Jalisco, Mexico. The seeds were dehulled, milled and passed through a 5 mm wire mesh. During milling, 188 mg/kg of ethoxyquin (ETQ 66.66%) were added.

A total of two hundred eighty-eight one-day-old male Ross 308 broilers were used. Eight groups of twelve broilers were fed one of three experimental diets (8 replicates per diet). The experimental diets were maize-soybean meal (MS), maize-soybean meal with enzymes (MSE), and maize-dehulled lupine seed with enzymes (MLE). The MLE diet was formulated using the chemical composition and AME values obtained in Experiment 1. Table 2.2 shows the composition of the experimental diets.

Table 2.2. Ingredient composition (% as fed) and calculated analysis (% dry matter) of the experimental diets used in the starter (S) and finisher (F) phases, Experiment 2.

Ingredient	**MS**		**MSE**		**MLE**	
	S	**F**	**S**	**F**	**S**	**F**
Maize	58.034	63.926	58.569	64.461	35.873	41.583
Soybean meal	35.816	30.285	35.715	30.184	---	---
Dehulled lupin	**---**	**---**	**----**	**----**	**51.756**	**47.518**
Soybean oil	1.923	1.740	1.743	1.560	7.000	6.916
Calcium carbonate	1.351	1.423	1.670	1.743	1.676	1.739
Dicalcium phosphate	1.918	1.363	1.328	0.773	1.061	0.509
L-Lysine	0.174	0.157	0.176	0.159	1.530	0.409
DL-Methionine	0.182	0.121	0.182	0.120	0.302	0.220
L-Threonine	0.017	---	0.018	---	0.152	0.078
L-Tryptophan	---	---	---	---	0.050	0.029
Sodium chloride	0.350	0.350	0.350	0.350	0.350	0.350
Vitamin-mineral premix[1]	0.085	0.085	0.085	0.085	0.085	0.085
Enzyme mixture[2]	---	---	0.010	0.010	0.010	0.010
Phytase[3]	---	---	0.005	0.005	0.005	0.005
Choline chloride	0.100	0.100	0.100	0.100	0.100	0.100
Pigment		0.400		0.400		0.400
Coccidiostat	0.050	0.050	0.050	0.050	0.050	0.050
Calculated analysis						
Metabolizable energy (MJ/kg)	12.56	12.80	12.58	12.80	12.58	12.78
Crude protein	21.06	19.04	21.06	19.04	21.01	19.02
Lysine	1.29	1.18	1.29	1.18	1.65	1.19
Methionine	0.53	0.44	0.53	0.44	0.53	0.40
Methionine + cystine	0.99	0.94	0.99	0.94	0.99	0.94
Calcium	0.99	0.89	0.99	0.89	0.99	0.89
Available phosphorus	0.49	0.45	0.49	0.45	0.49	0.45

MS, Maize-soybean meal diet. MSE, Maize-soybean meal diet with enzymes. MLE, Maize-dehulled seed of lupine diet with enzymes.

[1] Provided per kg of diet, vitamins: A, 12,000 UI; D3, 4,000 UI; K3, 5.0 mg; E, 0.05 UI; thiamine, 2.8 mg; riboflavin, 8 mg; pantothenic acid, 14.7 mg; pyridoxine, 3.6 mg; folic acid, 1.5 mg; niacin, 50 mg; biotin, 0.15 mg and cyanocobalamin, 0.03 mg. Minerals: Zn, 100 mg; Mn, 100 mg; Fe, 50 mg; Cu, 10 mg; Se, 0.3 mg and I, 1 mg.

[2] Contribution: Endo-1, 4-beta-xylanase (20,000 U/g), amylase (2,000 U/g) and protease (40,000 U/g).

[3] Contribution: 6-phytase (10,000 U/g).

Enzymes were added following the manufacturer's recommendations (DuPont-Danisco, Mexico City). The enzyme mixture (Axtra® XAP 101 TPT) was derived from *Bacillus subtilis*, *Trichoderma longibrachiatum* and *Bacillus licheniformis* and contained endo-1, 4-beta-xylanase 20,000 U/g, amylase 2,000 U/g and protease 40,000 U/g activities, and 6-phytase (Axtra® PHY 10000 TPT) was derived from *Trichoderma reesei*, 10,000 U/g activity.

Broilers were raised in 1 x 1.5 m pens (8 broilers/m^2) with wood shavings as litter. A lighting program of 23 h light:1 h dark (23L:1D) was used during the first four weeks, and from 29 until 44 days of age, the lighting program was 12L:12D. During the first week, the temperature in the poultry house was 33-35°C and was reduced successively 3°C per week. The experimental diets were divided into two phases: starter diet (1-27 days of age) containing 21% CP, 12.55 MJ ME/kg, 1.0% Ca and 0.50 available P; and finisher diet (28-44 days of age) containing 19% CP, 12.76 MJ ME/kg, 0.90% Ca and 0.45% available P. Diets were formulated to meet or exceed the nutritional recommendations for the Ross 308 line (Aviagen, 2014). Water and feed (mash form) were offered *ad libitum*.

Productive performance variables

Feed intake (FI; g/broiler/d), body weight (BW; g/broiler/d), body weight gain (BWG; g/broiler/d) and feed conversion (FC) were recorded weekly up to 41 days of age. Cumulative productive performance is also reported.

Size of digestive organs variables

On days 21 and 44, one broiler per replicate (weight close to the average of the pen) was selected, weighed and humanely killed by cervical dislocation. Small intestine and caeca lengths were obtained with a measuring tape on a wet cloth surface to avoid shrinkage. After cleaning the organs of residual tissues, the empty weight of crop, proventriculus, gizzard, small intestine and caeca were determined. Liver, spleen and pancreas weights were also recorded.

Welfare-related variables

On days 21 and 44, sixteen broilers per treatment (2 broilers per replicate) were randomly selected and humanely killed, according to the official Mexican standard NOM-033-SAG/ZOO-2014 (Norma Oficial Mexicana, 2015) by cervical dislocation to assess tibia breaking strength (TiBS) and tendon breaking strength (TeBS). Tibia and *gastrocnemius* tendon were dissected from the left leg and subjected to a breaking strength test using a Vernier Force Plate (Vernier Software & Technology, Beaverton, USA). The tibia was placed on an adjustable three-point loading system with a distance bone support of 60 mm, and a vertical force was applied at midpoint by a 2.54 cm fulcrum. The proximal and distal portion of each tendon were fastened with sandpaper and attached to the mounting brackets of the Vernier Force Plate. Breaking strength was recorded as the force in newtons (N) required to break the tibia or tendon.

On days 37 and 44, forty broilers per treatment were randomly selected to assess gait score, latency to lie down and *valgus/varus* angulation.

Gait score (GS) was evaluated simultaneously by two evaluators who scored each broiler according to the methodology described by Garner et al. (2002). Six score categories (0 to 5) were used: Briefly 0, broilers with a fluid locomotion and 5, completely lame broilers that cannot walk or stand.

Valgus/varus angulation (AngV) was evaluated with the scale described by Leterrier and Nys (1992). Depending on the angle size of tibia-metatarsus, four scores (0 to 3) were defined: 0, broilers that show no tibia-metatarsus angulation evident to the naked eye (tibia-metatarsus angle less than 10°) and 3, broilers with severe angulation (angle greater than 45°).

Latency to lie down (LLD) was evaluated according to the technique described by Berg and Sanotra (2003), which is based on the body contact of the broiler with water, which is an aversive experience for broilers. The broilers were placed in plastic containers with 3 cm water at 32°C, and the time elapsed in seconds was measured until the moment the broiler sat down. If the broiler remained standing after 600 seconds, the test was suspended; all tests were carried out by the same person. The broilers were evaluated individually without visual contact between them.

Statistical analysis

The SAS software was used to analyze the data. FI, BW, BWG and FC were analyzed as repeated measures, considering the pen as the experimental unit, using the MIXED procedure. AME and AMEn were analyzed with a completely random design of three treatments with eight replicates each, using the GLM procedure. For data on relative weights and lengths of organs, TiBS and TeBS; GS and VAng; and LLD a completely randomized experimental design in a 3 × 2 (diets and ages) factorial arrangement model, considering each killed broiler as the observational unit, was used and analyzed with GLM, FREQ and GLIMMIX, and MIXED procedures, respectively. Statistical difference was set at $P < 0.05$ and means were separated by using the Tukey's test.

2.4. Results

2.4.1. Experiment 1. Chemical composition and apparent metabolizable energy of whole and dehulled lupine seed

Dehulled seed had higher contents of ash, EE, CP, P, essential amino acids, AME and AMEn, but lower NDF, ADF and Ca than whole seed (Table 2.3).

2.4.2. Experiment 2. Productive performance, size of digestive organs and welfare-related variables in broilers fed dehulled lupine seed with enzymes

Productive performance

Cumulative feed intake was higher in broilers fed the MLE diet than in those fed the MS diet (Table 2.4). Final BW and cumulative BWG were higher in broilers fed the MSE diet than in those fed the MLE diet, and cumulative FC was higher in broilers fed the MLE diet than in broilers fed the other treatments.

Table 2.3. Chemical composition (% dry matter), essential amino acid content and metabolizable energy of whole and dehulled lupine seed (*Lupinus angustifolius* L. cv. Boregine).

Nutrient	**Whole seed**	**Dehulled seed**
Dry matter	91.09	90.58
Ash	3.81	3.92
Ether extract	5.42	6.47
Crude protein	25.01	31.10
NDF	28.99	13.02
ADF	21.85	8.18
Calcium	0.36	0.24
Phosphorus	0.61	0.77
Essential amino acids		
Methionine	0.16	0.23
Cystine	0.37	0.52
Lysine	1.22	1.43
Threonine	0.92	1.05
Tryptophan	0.24	0.31
Arginine	2.42	2.87
Isoleucine	0.99	1.23
Leucine	1.66	2.14
Valine	1.06	1.18
Histidine	0.68	0.85
Phenylalanine	0.99	1.21
GE (MJ/kg DM)	20.47	19.69
AME (MJ/kg DM)	5.93 b	8.80 a
AMEn (MJ/kg DM)	5.35 b	8.17 a

[ab] Different letters in a row indicate significant differences ($P < 0.05$). NDF, neutral detergent fiber. ADF, acid detergent fiber. GE, gross energy. AME, apparent metabolizable energy. AMEn, nitrogen-corrected AME.

Table 2.4. Cumulative productive performance of broilers (1-41 d of age) fed maize-soybean meal diet (MS), maize-soybean meal diet with enzymes (MSE) or maize-dehulled lupine (*Lupinus angustifolius* L. cv. Boregine) diet with enzymes (MLE).

Variable	**Diet**			**Standard error**
	MS	**MSE**	**MLE**	
Feed intake (g/broiler)	4,433.2 b	4,557.5 ab	4,641.4 a	33.7
Final body weight (g/broiler)	2,786.2 ab	2,885.9 a	2,694.4 b	25.2
Body weight gain (g/broiler)	2,740.3 ab	2,839.9 a	2,647.7 b	25.2
Feed conversion (g/g)	1.62 b	1.61 b	1.75 a	0.016

[ab] Different letters in a row indicate significant differences ($P < 0.05$).

Weekly productive performance is shown in Figure 2.1. As of the third week, feed intake was higher in the broilers fed the MLE diet than in those fed the MS diet. Broilers fed the MLE diet had body weight gains similar ($P > 0.05$) to those of broilers fed the MS and MSE diets in four of the six weeks of the experimental period. From the third week onwards, feed conversion of broilers fed the MLE diet was higher ($P < 0.05$) than that of broilers fed the other diets.

Size of digestive organs

The relative weight of proventriculus, gizzard and pancreas were affected ($P < 0.05$) by the diet. The broilers that received the MLE diet had higher ($P < 0.05$) relative weights of proventriculus and gizzard than the broilers that were fed the MS and MSE diets. The relative length of small intestine was longer ($P < 0.05$) in broilers fed the MLE diet than in broilers fed the MSE diet but similar ($P > 0.05$) to in broilers on the MS diet.

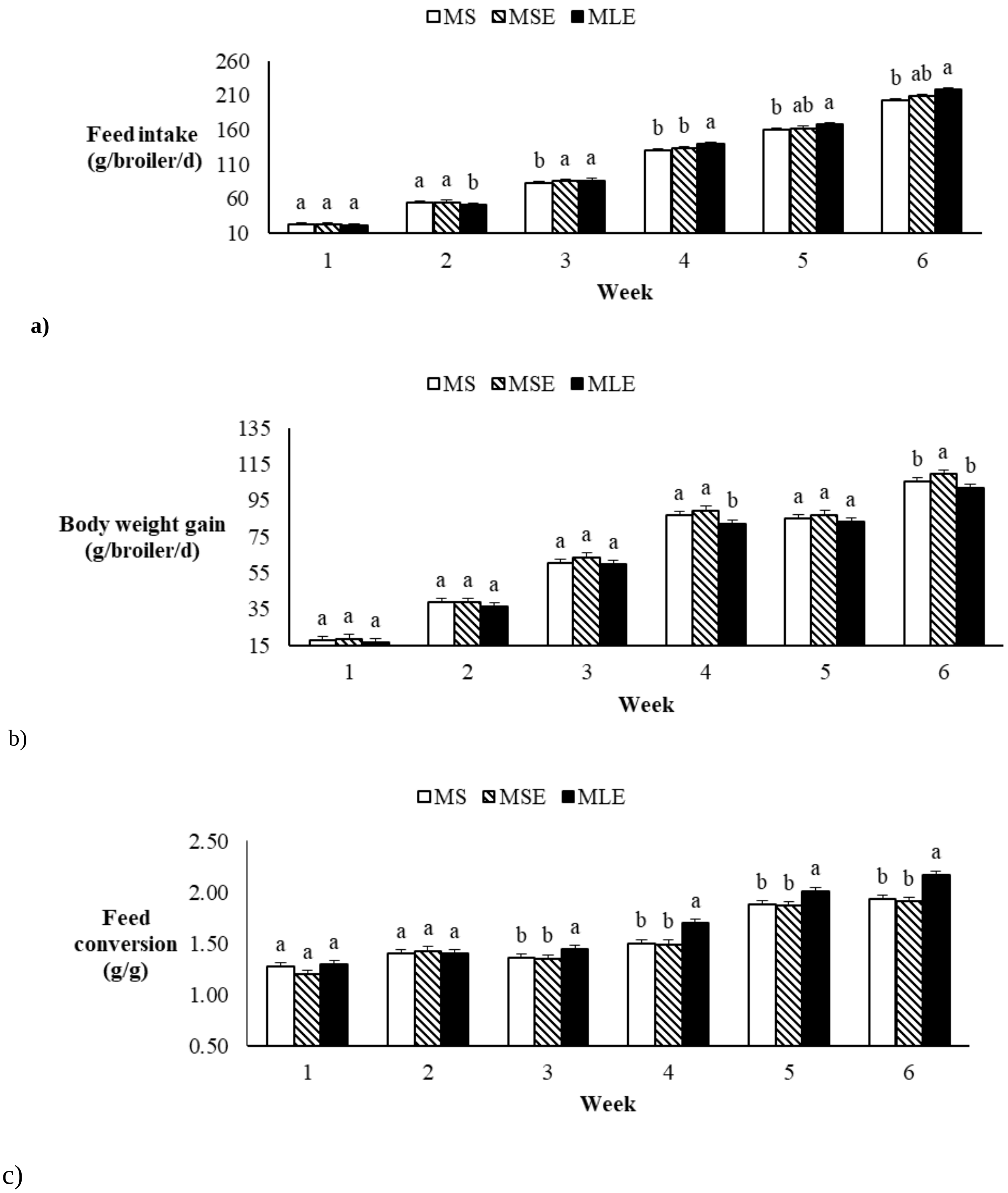


Figure 2.1. Weekly productive performance: a) feed intake, b) body weight gain and c) feed conversion of broilers fed maize-soybean meal diet (MS), maize-soybean meal diet with enzymes (MSE) or maize-dehulled lupine (*Lupinus angustifolius* L. cv. Boregine) diet with enzymes (MLE). ^{ab} Different letters on each bar (mean ± standard error), within each week indicate significant differences ($P < 0.05$).

With exception of relative spleen weight, age affected ($P < 0.05$) all parameters of digestive organ size (Table 2.5). It was found that the older the broilers, the lower the relative weights and lengths of organs. The diet × age interaction was significant ($P < 0.05$) only for relative pancreas weight (Figure 2.2), which was lower ($P < 0.05$) in broilers fed the MLE diet at 21 days, but similar ($P > 0.05$) in all treatments at 44 days of age.

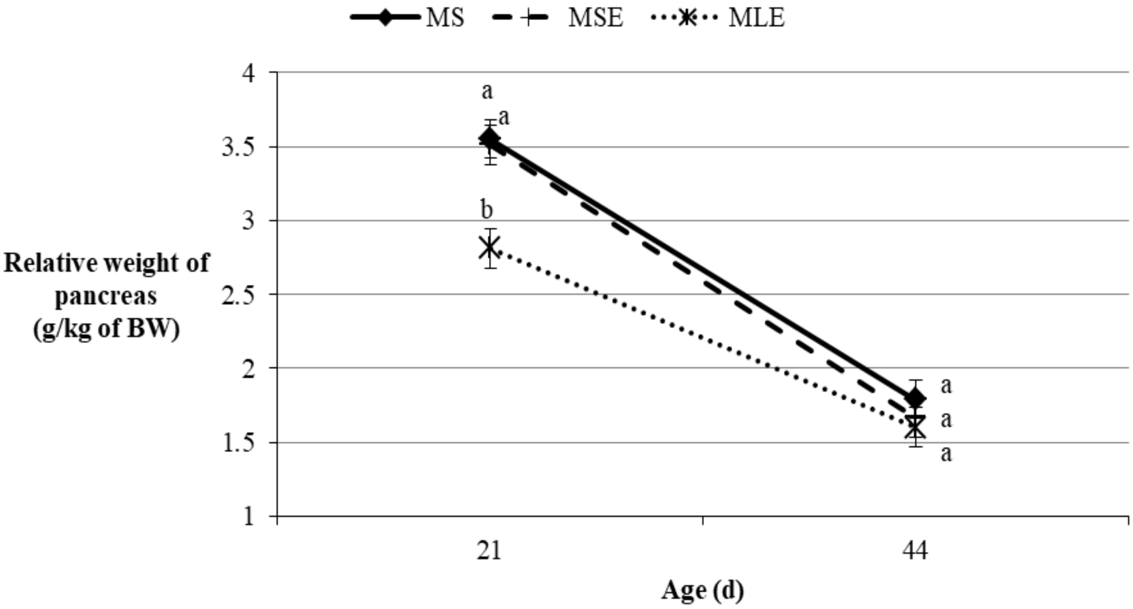


Figure 2.2. Relative weight of pancreas of 21- and 44-day-old broilers fed maize-soybean meal diet (MS), maize-soybean meal diet with enzymes (MSE) or maize-dehulled lupine (*Lupinus angustifolius* L. cv. Boregine) diet with enzymes (MLE). Values of P: diet= 0.0013; age < 0.0001; and diet*age= 0.0303. ^{ab} Different letters within each age (mean ± standard error) indicate significant differences ($P < 0.05$).

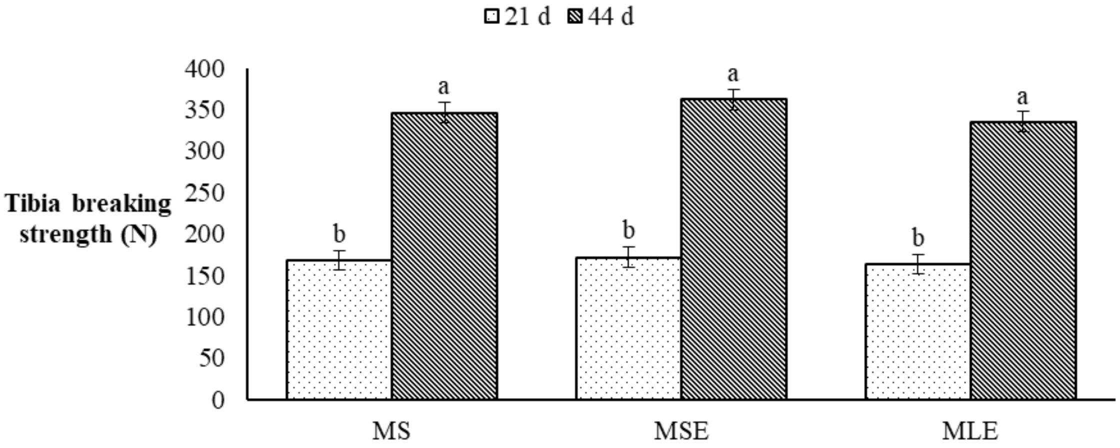
Table 2.5. Organ size of 21 and 44-day-old broilers fed maize-soybean meal diet (MS), maize-soybean meal diet with enzymes (MSE) or maize-dehulled lupine (*Lupinus angustifolius* L. cv. Boregine) diet with enzymes (MLE).

	Diet			**Age**		**Standard error**	**Values of P**		
	MS	**MSE**	**MLE**	**21**	**44**		**Diet**	**Age**	**Diet × age**
Relative empty weight (g/kg of body weight)									
Crop	2.55 a	2.54 a	2.84 a	2.91 a	2.38 b	0.072	0.0753	< 0.0001	0.9209
Proventriculus	3.60 b	3.52 b	4.29 a	4.76 a	2.85 b	0.164	< 0.0001	< 0.0001	0.2791
Gizzard	15.04 b	15.22 b	17.74 a	20.33 a	11.66 b	0.728	0.0019	< 0.0001	0.3556
Small intestine	30.51 a	30.89 a	32.42 a	38.94 a	23.61 b	1.316	0.5194	< 0.0001	0.7427
Caeca	3.72 a	3.51 a	3.93 a	4.35 a	3.09 b	0.126	0.1599	< 0.0001	0.6631
Relative organ weight (g/kg of body weight)									
Liver	21.76 a	22.47 a	21.95 a	25.75 a	18.38 b	0.595	0.5100	< 0.0001	0.1811
Spleen	1.00 a	1.06 a	1.08 a	0.99 a	1.11 a	0.036	0.6408	0.0935	0.2828
Pancreas	2.67 a	2.59 a	2.21 b	3.29 a	1.69 b	0.132	0.0013	< 0.0001	0.0303
Relative length (cm/kg body weight)									
Small intestine	114.67 ab	112.37 b	125.76 a	171.50 a	63.69 b	8.166	0.0301	< 0.0001	0.4807
Caeca	10.67 a	10.27 a	11.07 a	14.89 a	6.45 b	0.650	0.2891	< 0.0001	0.1161

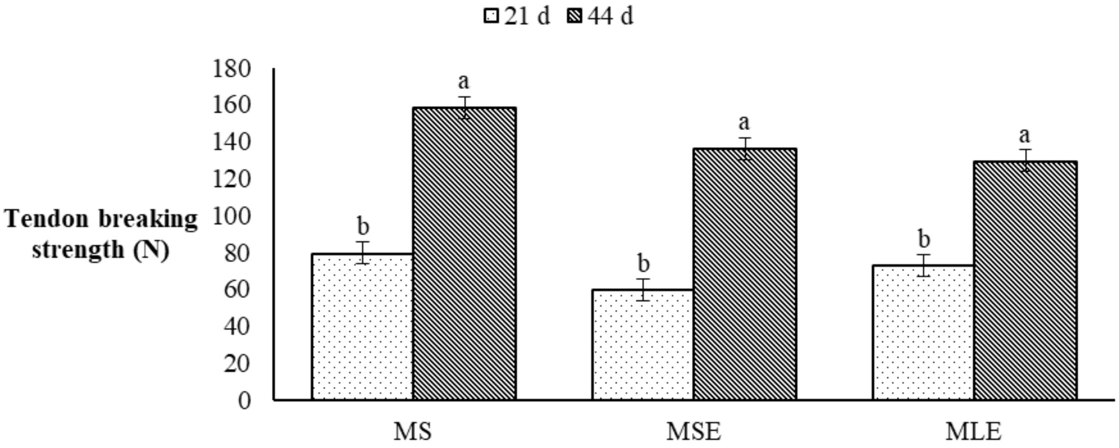
^{ab} Different letters in a row within either diet or age indicate significant differences ($P < 0.05$).

Welfare-related variables

The TiBS and TeBS were not affected by diet ($P > 0.05$; Figure 2.3). However, TiBS and TeBS were affected by age ($P < 0.05$); 44-day-old broilers had higher ($P < 0.05$) TiBS and TeBS than 21-day-old broilers. Diet × age interaction was not significant ($P > 0.05$).



a)



b)

Figure 2.3. Tibia (a) and tendon breaking strength (b) of broilers fed maize-soybean meal diet (MS), maize-soybean meal diet with enzymes (MSE) or maize-dehulled lupine (*Lupinus angustifolius* L. cv. Boregine) diet with enzymes (MLE). ^{ab} Different letters (mean ± standard error) indicate significant differences ($P < 0.05$).

The LLD was affected by diet ($P < 0.05$). Broilers that received the MLE diet took less ($P < 0.05$) time to lie down than those fed the MS and MSE diets (Figure 2.4). LLD of broilers was similar ($P > 0.05$) at 37 and 44 days of age. Diet × age interaction was not significant ($P > 0.05$).

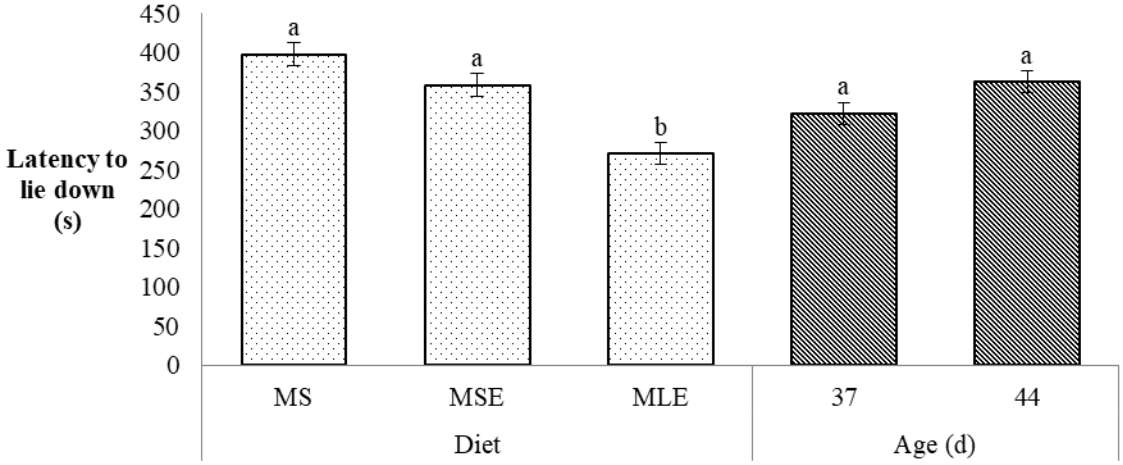


Figure 2.4. Latency to lie down of broilers tested at different ages and fed maize-soybean meal diet (MS), maize-soybean meal diet with enzymes (MSE) or maize-dehulled lupine (*Lupinus angustifolius* L. cv. Boregine) diet with enzymes (MLE). ^{ab} Different letters (mean ± standard error), within either diet or age indicate significant differences ($P < 0.05$).

The GS variable was affected by diet and age ($P < 0.05$). Broilers fed the MLE diet had less walking ability (higher frequency of lame broilers) than those on MS and MSE diets. Moreover, walking ability decreased with age (Tables 2.6). Diet × age interaction was not significant ($P > 0.05$). Similarly, AngV was affected by diet and age ($P < 0.05$). Broilers fed the MLE diet had higher AngV than those of the MS and MSE diets. Also, AngV increased with age (Tables 2.7). Diet × age interaction was not significant ($P > 0.05$).

Table 2.6. Effect of diet and age on gait score (%) of broilers fed maize-soybean meal diet (MS), maize-soybean meal diet with enzymes (MSE) or maize-dehulled lupine (*Lupinus angustifolius* L. cv. Boregine) diet with enzymes (MLE).

Gait score	**Diet**			**Age (d)**		**Values of P**		
	MS	**MSE**	**MLE**	**37**	**44**	**Diet**	**Age**	**Diet × Age**
0	31.25	27.50	16.25	43.33	6.67	< 0.01	< 0.01	0.30
1	35.00	32.50	25.00	43.33	18.33			
2	23.75	20.00	28.75	10.83	37.50			
3	10.00	17.50	17.50	0.83	29.17			
4	0.00	2.50	5.00	0.83	4.17			
5	0.00	0.00	7.50	0.83	4.17			

Briefly, a score of 0 denotes a bird with fluid locomotion and furled paw when it is raised and a score of 5 denotes a completely lame broiler that cannot walk or stand (Garner et al., 2002).

Table 2.7. Effect of diet and age on leg angulation score (%) of broilers fed maize-soybean meal diet (MS), maize-soybean meal diet with enzymes (MSE) or maize-dehulled lupine (*Lupinus angustifolius* L. cv. Boregine) seed diet with enzymes (MLE).

Angulation Score	**Diet**			**Age (d)**		**Values of P**		
	MS	**MSE**	**MLE**	**37**	**44**	**Diet**	**Age**	**Diet × Age**
0	56.25	43.75	28.75	70.83	15.00	< 0.01	< 0.01	0.78
1	36.25	45.00	43.75	25.83	57.50			
2	6.25	11.25	17.50	2.50	20.83			
3	1.25	0.00	10.00	0.83	6.67			

Valgus/varus angulation was evaluated according to the methodology described by Leterrier and Nys (1992): four scores (0 to 3) were defined: 0, normal broiler and 3, severe angulation (angle greater than 45°).

2.5. Discussion

The chemical composition data showed that lupine seed cultivar Boregine is a valuable source of protein (25% in whole seed). The protein content of whole lupine seeds was lower than what has been reported in other studies for *L. angustifolius*. This may be due to differences in cultivars; several authors have shown protein contents ranging from 26.5 to 35.8% (Vecerek et al., 2008; Nalle et al., 2011; Sujak et al., 2006), all with different cultivars.

It has been suggested that seed size has an influence on its composition. Kingwell (2005) points out that most of the protein in lupine seeds resides in the inner part and, because the seed coat constitutes a greater proportion in small seeds, large seeds tend to have more protein. Thus, it is likely that under the conditions where the crop was developed (Jalisco region), the seeds used in our study did not grow well enough and its protein content was reduced.

Nalle et al. (2011) evaluated whole seeds of three varieties of *L. angustifolius* and found amino acid contents similar to those of our study for isoleucine, phenylalanine and valine and slightly higher for arginine, histidine, leucine, lysine, methionine and threonine. In our study, arginine was the essential amino acid found in greater quantity in the lupine seed, a result that coincides with other studies. Nalle et al. (2011) found that arginine is the most abundant essential amino acid in seeds of this species. The high content of arginine may be of interest for reducing pulmonary hypertension in broilers raised at high altitude, through synthesis of nitric oxide (potent vasodilator produced from L-arginine). The central etiology in pulmonary arterial hypertension (ascites syndrome) is a hypoxemic condition, consequence of vasoconstriction and reduction of the vascular lumen which decreases blood flow. It has been suggested that the decrease in nitric oxide synthesis is responsible for vasoconstriction (Wideman et al., 2013). Sujak et al. (2006) indicate that glutamic acid, aspartic acid and arginine are the main amino acids in all proteins isolated from lupine. In our study, methionine and cystine were found in smaller amounts in lupine seed; according to Nadal et al. (2004), legume proteins are usually low in sulfur amino acids.

The results of our study showed that dehulling increased crude protein by six percentage units and decreased NDF and ADF (16.0 and 13.7 percentage units, respectively). According to Vecerek et al. (2008), the protein is mainly located in the cotyledons, while the fiber fractions are found in the hull, possibly explaining our results. The dehulling process of lupine seed increased all the essential amino acids in a range of 0.07 to 0.48 percentage units (dry matter basis).

The contents of calcium and phosphorus in whole and dehulled lupine seed were similar to those reported by Vecerek et al. (2008). Dehulling lupine seed decreased the calcium content and increased the phosphorus content. This is because calcium is deposited mainly in the hull, while phosphorus is found in the cotyledons (Vecerek et al. 2008).

The AME and AMEn values of whole seed were lower than those reported by Nalle et al (2011) for other varieties of *L.* a*ngustifolius*. The AMEn content in dehulled seed of our study was higher than that reported by Nalle et al. (2010) for dehulled seed of *L. angustifolius* Wallan variety. The dehulling process increased values of AME and AMEn by 48.4 and 52.7%, respectively. Based on these results, the nutritionist can increase the level of inclusion of dehulled lupine seed in diets for broilers when high-energy diets are needed. Nalle et al. (2010) report that dehulling reduces the content of non-starch polysaccharides and improves AME values.

It is important to point out that in the MLE diet the soybean meal was completely substituted by dehulled lupine seed. Accordingly, the results obtained in this study suggest that it is possible completely replace the soybean meal by dehulled lupine seed in broiler diets, which resulted in final weights of 93.4% with respect to the MSE diet and similar ($P > 0.05$) to the MS diet. In agreement with the above results, feed conversion increased around 0.13 - 0.14 g of feed per g of BWG in broilers fed the MLE diet with respect to those fed the other diets. The higher cumulative FI and FC observed in the broilers fed the MLE diet was possibly because the energy value of the dehulled lupine seed used in Experiment 2 was lower than that estimated in Experiment 1 since there was a difference in dehulling process efficiency (100% in the Experiment 1 vs 95% in the Experiment 2). It has also been reported that enzyme supplementation of lupine diets improves feed intake (Brenes et al., 2002).

An increment in relative weight of proventriculus (19 and 22%) and gizzard (18 and 17%) were observed in broilers on the MLE diet with respect to those on the MS and MSE diets. The greater relative weight of proventriculus and gizzard in broilers fed the MLE diet may be related to effects of the non-starch polysaccharides of the lupine seed. Similar results have also been reported in other studies. Brenes et al. (2002) observed an increase in the relative weight of crop, proventriculus, gizzard and duodenum when they included 30 or 45% of whole lupine seed in the diet.

Higher relative weight of proventriculus and gizzard may be of interest since an increase in the size of these organs, when the diet contains structural components, improves the digestive function through a longer retention time, which allows secretion of more hydrochloric acid and therefore pH is lower and grinding is better (Svihus, 2014).

Shirzadegan and Taheri (2017) observed higher relative weights of proventriculus and gizzard and better growth in broilers fed diets with 3% and 6% sources of insoluble fiber such as alfalfa meal and rice bran. The residual hulls (5%) of the lupine seed in our study could have an effect similar to that observed by these authors.

The greater relative liver weight found by Nalle et al. (2011) in broilers fed diets containing 20% of whole *L. angustifolius* seed was not observed in our study when higher levels of dehulled seed were included. Rubio et al. (2003) observed greater relative liver weight in broilers fed diets with 40% lupine seed, compared with a control diet (wheat-soybean), but that difference was eliminated by enzyme supplementation. Józefiak et al. (2007) suggested that liver size is dependent on gastrointestinal microflora and/or its fermentation products. Thus, it is possible that enzyme supplementation modulates microbial activity; this, however, should be tested.

The lower relative pancreas weight of broilers fed MLE diet is possibly linked to the negligible level of trypsin inhibitor activity, which has been reported in seeds of *L. angustifolius* (Nalle et al., 2011). A similar decrease in the relative pancreas weight of 21-day-old broilers was reported by Nalle et al. (2012) in broilers fed diets containing 20% whole *L. albus* cultivar Kiev mutant seed. In our study, however, at 44 days of age broilers fed the MLE diet had relative pancreas weight similar to that of broilers fed the MS and MSE diets. The meaning of this finding is unclear.

The relative length of the small intestine increased ($P < 0.05$) 11.9% in broilers fed the MLE diet in comparison with broilers on the MSE diet. Olkowski et al. (2005) reported 26.8 to 36.7% increases in the length of the different sections of the small intestine in broilers fed diets with 35% dehulled lupine seed. These authors explain the enlargement of the gastrointestinal tract in birds as an adaptive mechanism. The ancestors of domestic chickens included in their diet seeds containing high levels of indigestible material, such as NSP. For this reason, they speculate that chickens may have developed mechanisms by which increasing the intestine size increases its absorption capacity to extract maximum nutritional benefit from a poor quality diet.

Welfare-related variables (poor walking ability, less latency to lie down and more leg angulation) observed in broilers fed the MLE diet may have been due to higher cumulative FI and FC, which increase the excreta output and consequently the presence of wet cakey litter in their pens. Wet litter has been linked to poor walking ability (Su et al., 2000) and valgus deformity (Dawkins et al., 2004). The wet cakey litter observed in pens of broilers fed MLE diet may have been caused by the NSP in the lupine seed. Caprita et al. (2010) point out that the main NSP of lupine seeds is a complex branched chain structure containing long β-(1-4)-D-galactose side chains attached to a pectin-like main chain of rhamnose and galacturonic acid linked by β-(1-4) and α-(1-2) bonds, respectively, and α-(l-5)-L-arabinose side chains. Banch Knudsen (2001) indicates that seeds with high pectin content have higher water retention capacity. Therefore, in future research it is highly recommended to include pectinases, among other enzymes, to improve the litter conditions and welfare-related variables. Results of this study indicate that the enzyme mixture added to the MLE diet did not improve animal welfare-related variables.

2.6. Conclusions

The dehulling process of lupine seed improves the content of protein and apparent metabolizable energy and reduces fiber content. Therefore, it is possible completely replace soybean meal by dehulled lupine seed with enzymes in broiler diets and obtain similar body weights. However, it increases feed conversion (a higher feed intake is required) and deteriorates welfare-related variables. In further studies, evaluating increased enzyme concentrations and types is recommended to improve the results obtained in this study.

2.7. References

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CAPÍTULO III

TOTAL SUBSTITUTION OF SOYBEAN MEAL BY DEHULLED LUPINE (*Lupinus angustifolius* L.) SEED AND ENZYME SUPPLEMENTATION IN STARTER DIETS FOR BROILERS

3.1. Abstract

The objective of this study was to determine the effect of the total substitution of soybean meal for dehulled lupine seed and enzyme supplementation in starter diets for broilers on productive performance, size of digestive organs, ileal digestibility and apparent metabolizable energy corrected by nitrogen (AMEn). The experiment was conducted with 252 male broiler chickens (Ross 308) divided into four groups, each fed one of the experimental diets, 7 replicates with 9 birds each. Chickens were fed either maize and soybean meal (M-S) diet or maize and dehulled lupine seed (M-L) diet as main ingredients, either with (E) or without enzyme addition. The enzymes added in the combination were xylanases, β-glucanases and proteases. Chickens fed the M-L diets showed the lowest weight gain and feed intake, as well as, ileal digestibility and AMEn of the diet, and the highest feed conversion, relative weight of gizzard, relative weight and length of the small intestine and the caeca. Enzyme supplementation increased feed intake, weight gain and the AMEn content of the diet in chickens fed the M-SE diet. However, in chickens fed the M-LE diet, enzyme supplementation did not improve in weight gain, ileal digestibility, or AMEn content of the diet. It is concluded that substitution of soybean meal by dehulled lupin seed decreases ileal digestibility, AMEn content of the diet, and productive performance, while it increases digestive organs size. The mixture of enzymes used in this study does not improve digestibility nor AMEn content of the diet with dehulled lupine seed.

Key words: legume; ileal digestibility; size of digestive organs; apparent metabolizable energy.

3.2. Introduction

Soybean meal is the most widely used protein source in chicken diets worldwide (Stein et al., 2008). Its acceptability is due to a relatively high protein content, an amino acid profile that complements that of cereals, and high digestibility (Ravindran et al., 2014). However, in recent years, the cost of this ingredient has increased (Florou-Paneri et al., 2014) and has promoted interest in studying sources of vegetable protein that can replace soybean meal in animal nutrition (Kubiś et al., 2018). Lupine seed as an alternative protein source in poultry diets has received increasing attention in countries where soil and climate conditions are not suitable for soybean production (Kaczmarek et al., 2016). Seeds of modern varieties of lupine (*Lupinus angustifolius* L.) have high protein content (35%) and low concentrations of alkaloids; thus, it is believed that it can substitute soybean meal in diets for chickens (Petterson, 2000). However, the concentration of non-starch polysaccharides (NSP) is almost twice as high in lupines as in other vegetable protein sources (Bach Knudsen, 1997), restricting its use in chicken diets. Since there are no endogenous enzymes that degrade NSP in the digestive system, it is thought that lupine NSP can affect feed intake and digestibility (Naveed et al., 1999).

Nalle et al. (2011) suggest that the removal of the hull and the use of exogenous enzymes would allow inclusion of high amounts of lupine seed in broiler diets. Studies have been conducted to evaluate the effect of exogenous enzyme supplementation in *Lupinus angustifolius* seed-based diets for chickens; however, most of the experiments were carried out using whole seed (Smulikowska et al., 2014) and, in smaller numbers, using dehulled seed (Annison et al., 1996; Hughes et al., 2000). The results have been variable. Some authors report no effect, while others indicate a significant increase in digestibility, apparent metabolizable energy (AME) and productivity (Hughes et al., 2000, Steenfeldt et al., 2003). The effect of dehulling has also been reported by Nalle et al. (2010), who indicated that removal of lupine seed hulls decreases NSP content and increases AME content and amino acid concentrations.

In chickens, technical and economic results are determined during the starter phase, therefore, efficient performance must be guaranteed from the beginning, so that birds can express their growth potential (Barbé et al., 2017). Nalle et al. (2011) concluded that whole lupine seed can be included at a level of 20% in starter diets for broilers with no detrimental effect on productive performance. However, total substitution of soybean meal for dehulled lupine seed in this phase has not been evaluated. In this sense, the hypothesis of this study was that removal of the seed hull and enzyme supplementation make it possible to completely replace the soybean meal with dehulled lupine seed in starter diets for broilers without affecting productive performance, size of digestive organs, ileal digestibility or AME corrected by nitrogen (AMEn) value of the diet.

The objective of this research was to evaluate the effect of total substitution of soybean meal by dehulled lupine (*L. angustifolius*) seed and enzyme supplementation in starter diets for broilers on productive performance, size of digestive organs, apparent ileal digestibility and AMEn of the diet.

3.3. Materials and methods

The experiment was carried out in accordance with the regulations for use and care of animals intended for research at the Colegio de Postgraduados. The experiment was carried out in the poultry facilities of the Colegio de Postgraduados, Campus Montecillo, located in Texcoco, State of Mexico, Mexico, at 19° 29' N, 98° 54' W, and 2,247 m altitude.

3.3.1. Diets and enzymes

Lupine seed, cultivar Boregine, was produced with irrigation during the autumn-winter cycle (2015-2016) in Jalisco, Mexico, at 20° 43' N, 103° 23' W and 1,560 m altitude. The seeds were dehulled and ground using a 5 mm screen.

The chemical composition of dehulled lupine seed is presented in Table 3.1. Composition of the diets and the calculated analysis is shown in Table 3.2. Chickens were fed either maize and soybean meal (M-S) diet or maize and dehulled lupine seed (M-L) diet as main ingredients, either with (E) or without enzyme addition. Each diet was supplemented with essential amino acids to meet or exceed the recommended requirements (Aviagen, 2017) for broilers.

Table 3.1. Chemical composition (% dry matter) of dehulled lupine seed (*Lupinus angustifolius* L. cv. Boregine) used in the study.

Nutrient	**%**
Dry matter	90.58
Ash	3.92
Ether extract	6.47
Crude protein	31.10
Neutral detergent fiber	13.02
Acid detergent fiber	8.18
Calcium	0.24
Phosphorus	0.77

Table 3.2. Composition of ingredients (%) and nutritional analysis of experimental diets.

Ingredient	**M-S**	**M-SE**	**M-L**	**M-LE**
Ground maize	58.777	58.620	35.782	35.825
Soybean meal	34.307	34.336	---	---
Dehulled lupin	**---**	**---**	**51.067**	**50.863**
Vegetable oil	1.593	1.646	7.000	7.000
Calcium carbonate	1.185	1.185	1.187	1.187
Dicalcium phosphate	2.226	2.226	1.958	1.961
L-lysine	0.670	0.669	1.450	1.529
DL-methionine	0.429	0.429	0.571	0.573
L-threonine	0.215	0.215	0.335	0.335
L-tiyptophan	---	---	0.051	0.052
Salt	0.300	0.300	0.300	0.300
Premix of vitamins y minerals[1]	0.300	0.300	0.300	0.300
Enzymes[2]	----	0.075	----	0.075
Calculated anlysis (%)				
ME (MJ · kg^{-1})	12.55	12.55	12.55	12.55
Crude protein	21.00	21.00	21.00	21.00
Lysine	1.44	1.44	1.52	1.57
Methionine + cystine	1.07	1.07	1.08	1.08
Calcium	1.00	1.00	1.00	1.00
Phosphorus available	0.50	0.50	0.50	0.50

M-S: Diet based on maize-soybean meal. M-SE: M-S with enzymes. M-L: Diet based on maize-Dehulled lupin seed. M-LE: M-L with enzymes.

[1] Contributes per kilogram of feed: vitamin A, 12,000 IU; vitamin D3, 1,000 IU; vitamin E, 60 IU; vitamin K, 5.0 mg; vitamin B2, 8.0 mg; vitamin B12, 0.030 mg; pantothenic acid, 15 mg; niacin, 50 mg; folic acid, 1.5 mg; hill, 300 mg; biotin, 0.150 mg; thiamine, 3.0 mg. Minerals: Fe, 50.0 mg; Zn, 110 mg; Mn, 100 mg; Cu, 12.0 mg; Se, 0.3 mg; I, 1.0 mg.

[2] Enzymes supplemented: xylanase 2000 FXU · g^{-1} (150 mg · kg^{-1}), glucanase 50 FBGU · g^{-1} (400 mg · kg^{-1}) and protease 75,000 PROTU · g^{-1} (200 mg · kg^{-1}).

The enzymes added in the combination treatment were xylanases, β-glucanases and proteases (DSM Nutritional Products Ltd.). Enzyme activity reported by the supplier was 2,000 FXU·g^{-1}, 50 FBGU·g^{-1} and 75,000 PROTU·g^{-1} for xylanase, β-glucanase and protease, respectively.

3.3.2. Chickens, housing and management

The experiment was carried out with 252 one-day-old male chicks (Ross 308) distributed in 28 cages (42 × 120 cm) and arranged in batteries, with nine birds housed per cage (experimental unit). Each cage had a 1 cm^2 wire mesh floor, a channel-shaped feeder and a waterer. The batteries were located in a closed poultry facility. The lighting schedule was 23 h of light and 1 h of darkness during the experiment (1-25 days of age) and temperature was gradually reduced 3 °C per week, from 33 °C the first week to 27 °C the third week. Feed and water were available at all times during the experimental period.

3.3.3. Productive performance

Cumulative productive performance (feed intake, weight gain and feed conversion) from 1 to 21 days of age is reported.

3.3.4. Development of the digestive system

On day 21, seven birds weighing close to the average weight of each treatment were selected, weighed and killed by stunning and bleeding following the official Mexican standard NOM-033-SAG / ZOO-2014 (2015). Small intestine and ceaeca length were measured on a wet cloth surface to avoid contraction. After removing the mesentery adhered to each section of the digestive system, the empty weights of crop, proventriculus, gizzard, small intestine and ceaeca were determined. Weights of liver, spleen and pancreas were also recorded. Subsequently, the lengths (cm·kg^{-1} of body weight) and relative weights (g·kg^{-1} of body weight) were calculated.

3.3.5. Collection of excreta and content of small intestine

To measure apparent metabolizable energy, titanium dioxide (3 g·kg^{-1}) was added to the diets as a non-digestible marker. Experimental diets with titanium dioxide were provided to chickens as follows: from 18 to 21 days (adaptation), from 22 to 24 (excreta collection) and 24 to 25 (collection of ileal content).

On days 22 to 24, the tray under each cage was used to collect excreta twice a day; all the chickens in the seven experimental units of each treatment were included. The excreta collected from each cage during the three days were mixed and a pooled sample was analyzed. Contaminants such as feathers and scales were carefully removed before moisture content of excretas was determined and the excreta were stored in closed plastic containers at -20 °C and lyophilized.

On day 25, 42 chickens per treatment were humanely killed by stunning and bleeding in accord with the official Mexican standard NOM-033-SAG / ZOO-2014 (2015). Immediately after bleeding, the abdomen was sectioned to expose the digestive system and collect the total content of the ileum (from 2 cm after Meckel’s diverticulum to 2 cm before the ileo-cecal junction). The ileal content obtained from the chickens in each experimental unit was mixed. The material was placed in plastic containers and stored at -20 °C for later lyophilization.

3.3.6. Laboratory analysis

Samples of dried ileal chyme, dry excreta and diets were ground and passed through a 0.5 mm sieve. Dry matter (DM), crude protein (CP) or N × 6.25, ash, organic matter (OM) and ether extract (EE) were estimated in ileal chyme and diets following AOAC methods (1990). To estimate apparent metabolizable energy corrected by nitrogen (AMEn), gross energy (GE) and nitrogen content were determined in excreta and diets. GE was determined with an isoperibolic calorimeter (PARR 1266, Parr Instrument Company, Moline, Illinois, USA). Titanium dioxide (TiO_2) was determined in ileal chyme and excreta using the procedure described by Myers et al. (2004).

3.3.7. Calculations

Apparent ileal digestibility (AID) of DM, OM, CP and EE and content of AMEn were calculated by the ratio of titanium dioxide with respect to the content of the nutrient in question in the diet, chyme or excreta, using the following formulas:

AID DM = $[[(1 / TiO_2)_{diet} - (1 / TiO_2)_{chyme}] / (1 / TiO_2)_{diet}] \times 100$; AID Nutrient = $[[(Nu / TiO_2)_{diet} - (Nu / TiO_2)_{chyme}] / (Nu / TiO_2)_{diet}] \times 100$. Where contents of TiO_2 and nutrients (Nu) in the diet and chyme are given in $g \cdot kg^{-1}$.

AMEn (MJ / kg) = $GE_{diet} - [GE_{excreta} \times (TiO_{2diet} / TiO_{2excreta})] - 0.0344 \times \{\%N_{diet} - [\%N_{excreta} \times (TiO_{2diet} / TiO_{2excreta})]\}$. Where GE represents gross energy ($MJ \cdot kg^{-1}$) and N represents nitrogen (%). AMEn was corrected with a zero nitrogen balance using 0.0344 $MJ \cdot g^{-1}$ N retained.

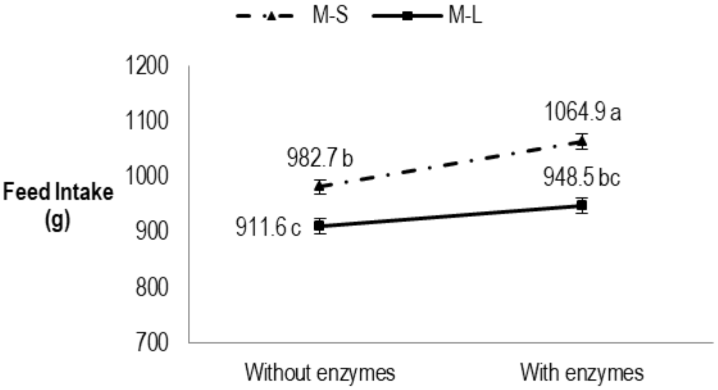
3.3.8. Experimental design and statistical analysis

Data were analyzed with a completely randomized design in a 2 × 2 factorial arrangement [two diets: M-S and M-L and two levels of enzyme: with (E) and without]. Each cage (nine birds) was the experimental unit. Data were analyzed by analysis of variance using the GLM procedure of SAS. Statistical differences were established at $P < 0.05$ and the means were separated by the Tukey test.

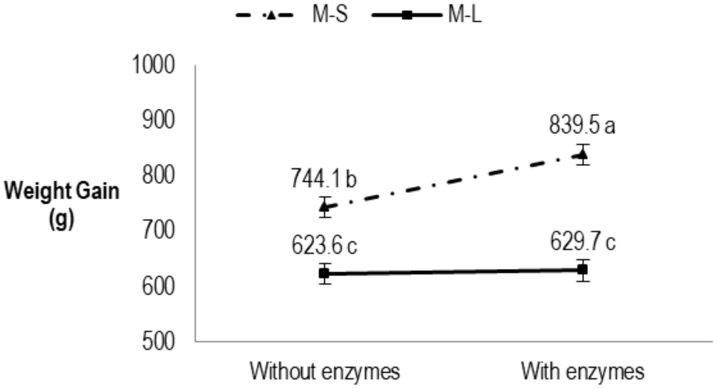
3.4. Results

3.4.1. Productive performance

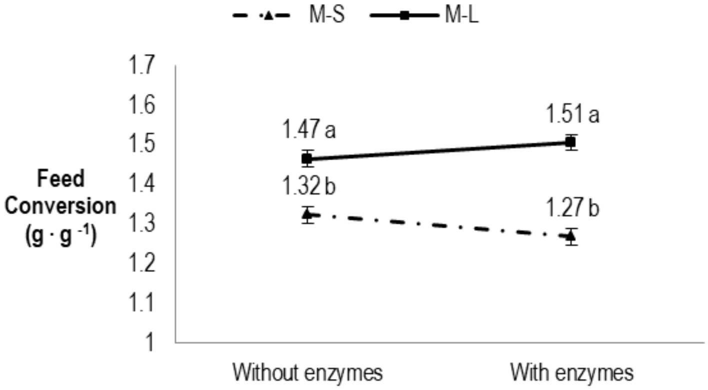
The effect of including dehulled lupine seed and enzyme supplementation on cumulative productive performance is presented in Figure 3.1. The main effects were statistically significant for feed intake; birds fed the M-L diets showed lower values in this variable. The enzyme effect was higher in birds fed the M-S diet than in those fed the M-L diet. It is important to point out that the feed intake of birds fed the M-LE diet was similar to that observed with the M-S diet without enzymes. The Diet × Enzyme interaction was not significant ($P > 0.05$) for feed intake, but it was significant ($P < 0.05$) for weight gain and feed conversion. Enzyme supplementation increased weight gain in chickens fed diets based on maize-soybean meal; however, in chickens fed M-L diets, enzyme supplementation did not improve these variables. Weight gain was lower ($P < 0.05$) in chickens fed the M-L diets. Feed conversion was higher ($P < 0.05$) in chickens fed the M-L diets than in those fed the M-S diets.



P values: Diet < 0.01, Enzyme < 0.01, Diet × Enzyme = 0.17



P values: Diet < 0.01, Enzyme < 0.01, Diet × Enzyme = 0.02



P values: Diet < 0.01, Enzyme = 0.67, Diet × Enzyme < 0.01

Figure 3.1. Cumulative productive performance (from day 1-21 of age) of broiler chickens fed diets based on maize-soybean meal (M-S) or maize-dehulled lupine seed (M-L) without or with enzymes. ^{ab} Different letters indicate significant differences ($P < 0.05$).

3.4.2. Size of digestive system organs

Size of digestive organs is shown in Table 3.3.

Table 3.3. Size of digestive organs at 21 days of age of broilers fed diets based on soybean meal (M-S) or dehulled lupine seed (M-L) as the main source of protein.

	Diets		**SE**	**P values**		
	M-S	**M-L**		**PS**	**E**	**PS × E**
Empty relative weight (g · kg^{-1} body weight)						
Crop	3.50	3.95	0.302	0.47	0.87	0.17
Proventriculus	5.17	5.51	0.169	0.34	0.83	0.56
Gizzard	16.40 b	19.73 a	0.550	< 0.01	0.85	< 0.01
Small intestine	34.46 b	46.76 a	1.689	< 0.01	0.77	0.34
Caeca	4.18 b	6.15 a	0.251	< 0.01	0.18	0.40
Relative weight (g · kg^{-1} body weight)						
Liver	23.54	26.07	0.794	0.12	0.60	0.55
Spleen	1.04	1.15	0.055	0.31	0.44	0.35
Pancreas	2.94	2.85	0.088	0.64	0.71	0.24
Relative length (cm · kg^{-1} body weight)						
Small intestine	157.70 b	225.79 a	8.181	< 0.01	0.57	0.70
Caeca	14.88 b	19.08 a	0.582	< 0.01	0.28	0.42

[ab] Different letters in a row indicate significant differences ($P < 0.05$). SE: Standard error, PS: Protein source, E: Enzyme supplementation.

Relative weight of gizzard, small intestine and caeca and relative length of small intestine and caeca were higher ($P < 0.05$) in chickens fed the M-L diets than in those fed the

M-S diets. Enzyme supplementation did not affect ($P > 0.05$) digestive organ size. The Diet × Enzyme interaction was significant only for the relative weight of the gizzard. In diets without enzymes, the relative weight of the gizzard was higher ($P < 0.05$) with M-L than with the M-S diet. However, in chickens fed diets supplemented with enzymes (M-SE and M-LE) relative gizzard weights were similar ($P > 0.05$, Figure 3.2).

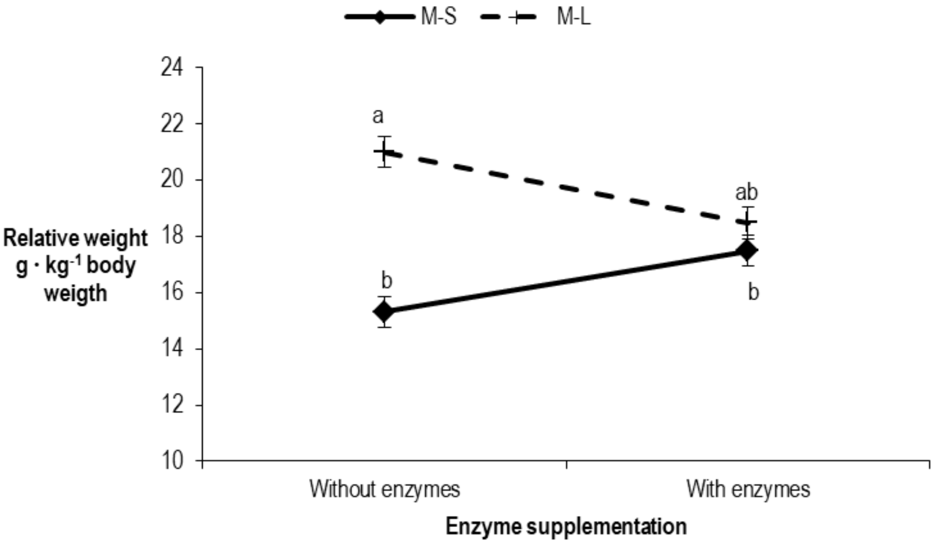


Figure 3.2. Relative weight of empty gizzard of broiler chickens fed diets based on maize-soybean meal (M-S) or maize-dehulled lupine seed (M-L); without enzymes or with enzymes. [ab] Different letters indicate significant differences ($P < 0.05$).

3.4.3. Excreta dry matter

Chickens fed the M-L diets showed significant reduction ($P < 0.05$) in excreta DM content (Figure 3.3). Enzyme supplementation and the Diet × Enzyme interaction were not significant ($P > 0.05$).

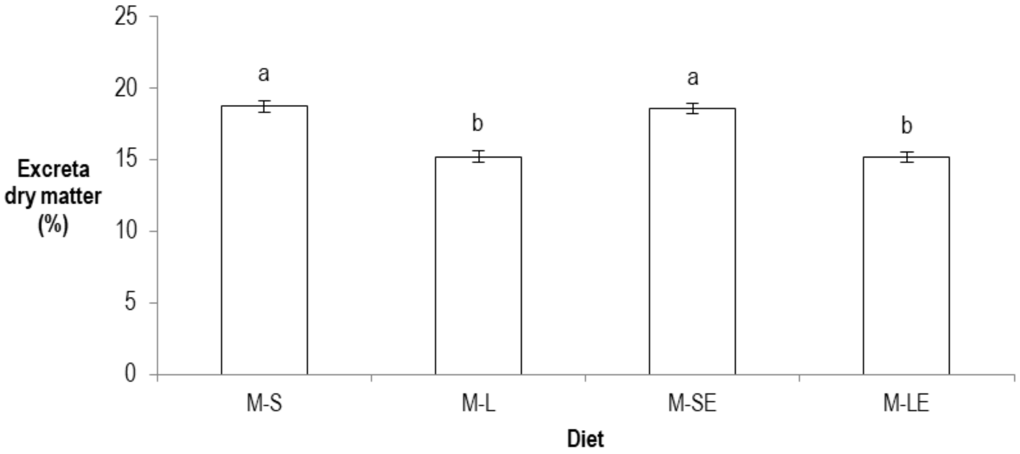


Figure 3.3. Dry matter from the excreta of broiler chickens fed diets based on maize-soybean meal (M-S) or maize-dehulled lupine seed (ML) without or with enzymes (M-SE, M-LE). [ab] Different letters in each bar (mean ± standard error), indicate significant differences ($P < 0.05$).

3.4.4. Apparent ileal digestibility and apparent metabolizable energy of the diet

Ileal digestibility of DM, MO and EE, as well as AMEn content, decreased ($P < 0.05$) in chickes fed the M-L diets, but they were not affected ($P > 0.05$) by enzyme supplementation (Table 3.4). Ileal digestibility of CP was higher ($P < 0.05$) in M-L diets than in M-S diets. The Diet × Enzyme interaction was only significant ($P > 0.05$) for the AMEn variable. In chickens fed the M-SE diet AMEn content increased, while in chickens fed the M-LE diet the AMEn value did not improve (Figure 3.4).

Table 3.4. Apparent ileal digestibility (%) and apparent metabolizable energy corrected by nitrogen (AMEn) in broiler chickens fed diets based on soybean meal (M-S) or dehulled lupine seed (M-L) as the main source of protein.

	Diets		**SE**	**P values**		
	M-S	**M-L**		**PS**	**E**	**PS × E**
Dry matter	70.7 a	48.9 b	2.11	< 0.01	0.43	0.80
Organica matter	72.2 a	49.5 b	2.20	< 0.01	0.34	0.82
Protein	80.9 b	83.0 a	0.32	< 0.01	0.42	0.42
Ethereal extract	89.3 a	84.5 b	0.79	< 0.01	0.32	0.17
AMEn (MJ · kg^{-1})	13.11 a	10.75 b	0.252	< 0.01	0.11	0.03

[ab] Different letters in a row indicate significant differences ($P < 0.05$). SE: Standard error, PS: Protein source, E: Enzyme supplementation.

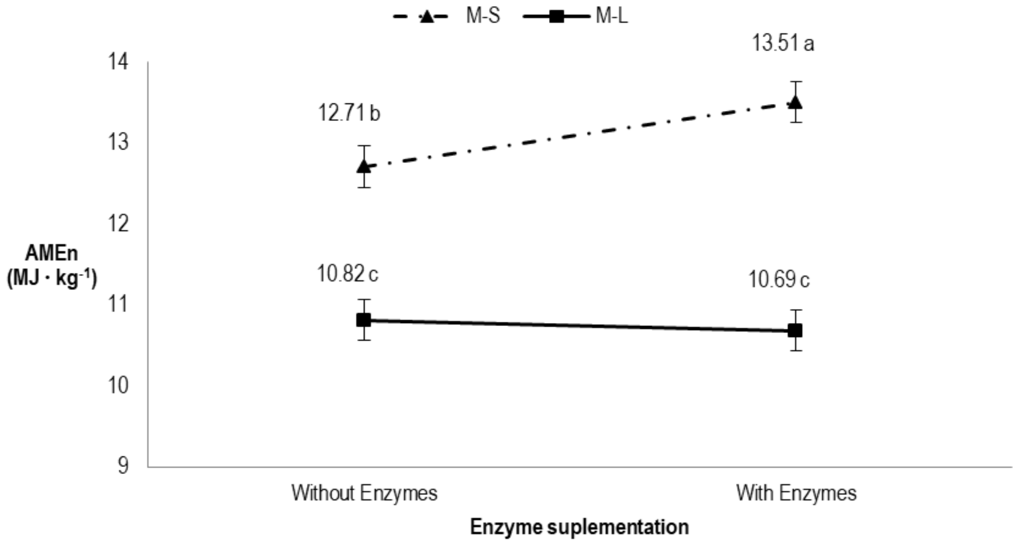


Figure 3.4. Value of apparent metabolizable energy corrected by nitrogen (AMEn) of the diet in broiler chickens fed diets based on maize-soybean meal (M-S) or maize-dehulled lupine seed (M-L) without or with enzymes. [abc]Different letters indicate significant differences ($P < 0.05$).

3.5. Discussion

Chickens fed the maize-dehulled lupine seed diet (M-L, 51% dehulled lupine seed) showed a reduction of 7.2% in feed intake and 16.2% in weight gain and an increase of 11.4% in feed conversion compared with chickens fed M-S diets. These results are consistent with other investigations where dehulled lupine seed was used in diets for broilers, such as those reported by Olkowski et al. (2001), who evaluated the effect of including dehulled lupine (*Lupinus angustifolius* cv. Troll) seed (35%) in broiler (1 to 21 days of age) diets and observed a decrease in feed intake (26.8%) and weight gain (31.5%) compared with chickens fed a diet based on maize-soybean meal. Hughes et al. (2000) observed a decrease in growth and an increase in feed conversion, when they included 10 or 15% lupine kernel isolate (with 56% of NSP) in the diet. The lower productive performance observed in chickens fed the M-L diets is explained by decreases in apparent ileal digestibilities of dry matter, organic matter and ether extract of approximately 30.8, 31.4 and 5.4%, respectively, as well as by the lower AMEn content determined in the diets (2.36 MJ·kg^{-1} reduction, 18.0%) relative to the M-S diets.

The high content of NSP in dehulled lupine seed probably decreased digestibility and AMEn content of the diet and reduced productive performance of broilers. Steenfeldt et al. (2003) reported that in chickens fed diets with 20% lupine seed containing high NSP (45%), apparent digestibility of organic matter decreased by approximately 10% and 0.9 MJ·kg^{-1} in AMEn content in comparision with the control diet. The NSP are indigestible in the upper intestinal tract of birds due to absence of the specific endogenous enzymes (Annison et al., 1996; Smits and Annison, 1996). Two alternatives have been proposed to explain the antinutritional effect of NSP in diets for broilers: encapsulation by the NSP coat, which hinders access of digestive enzymes to starch, fat and protein, and the presence of NSP in the intestine increases the viscosity of intestinal contents (Caprita et al., 2010). As NSP increases chyme viscosity, diffusion of enzymes, substrates and products decreases (Petterson and Aman, 1989), thus limiting the mixture of nutrients with pancreatic enzymes and bile acids. In addition, a diet high in NSP can cause a decrease in the length, width and surface area of the villi, with a consequently negative effect on nutrient absorption (Mathlouthi et al., 2002). In this way, NSP can reduce digestion and absorption of nutrients due to its physical-chemical effect in the intestinal tract (Caprita et al., 2010). However, viscosity was not evaluated in the present study, so it is necessary to examine it in the future.

An important finding of our study is that protein digestibility in chickens fed M-L diets was slightly but significantly higher (2.6%) than that of chickens fed the M-S diets. This indicates that the DLS protein is as digestible as, or more digestible than, soybean meal protein. Steenfeldt et al. (2003) observed that the protein in diets with 20% lupine seed was digested to the same extent as the protein in the diets with soybean meal, but they found an average apparent ileal digestibility of 75%. In our study we obtained higher values of apparente ileal digestibility (83%).

Supplementation with enzymes in the M-SE diet increased cumulative feed intake by 8.4% and weight gain by 12.8%, but productive performance of chickens fed the M-LE diet did not improve with enzymes. Enzyme supplementation showed no effect on ileal digestibility. AMEn content, however, increased by 0.797 MJ·kg^{-1} (6.3%) only in the M-SE diet, which explains the better productive performance of chickens fed that treatment. Steenfeldt et al. (2003) did not observe effects of adding enzymes to diets with lupine seed on the apparent digestibility coefficients of the organic matter measured in the ileum, but addition of other enzymes increased the apparent digestibility of organic matter in the total digestive tract. The fact that the enzymes used in our study did not improve digestibility or AMEn content of the M-LE diet indicates that they were probably not effective in degrading the NSP of dehulled lupin seed. It is known that the main NSP of the cotyledon of *Lupinus angustifolius* seed is a complex polysaccharide of the highly branched pectic type consisting of a main chain of rhamnogalacturonans (chains (1-4) -α-D-galacturones that are interrupted by insertion of residues of (1-2) -β-L-rhamnose) with long side chains of arabinanos and galactanos (composed of D-galactose, L-arabinose, D-xylose, L-fucose and D-glucuronic acid) (Cheetham et al., 1993). Therefore, it is inferred that the enzymes used in our study, such as the β-glucanases that effectively degrade NSP of cereals, which have simpler structures, were not appropriate for degrading the NSP of dehulled lupin seed. To have an effect on these NSP, the enzymes must perform a series of different activities (Hughes et al., 2000). Ali et al. (2009) observed that a combination of pectinases (pectin methyl esterase and polygalacturonase) increased hydrolysis of polysaccharides, reduced viscosity of the digesta and improved feed conversion and apparent metabolizable energy of the chicken diet with 10 and 20% dehulled lupine seed. In this regard, Ali et al. (2009) comment that a commercial preparation of pectinases should be developed for its application to lupine diets for broilers.

The increase in relative weight of the gizzard, small intestine and caeca in chickens fed the M-L diets was 20.3, 35.7 and 47.1%, respectively, while the relative length of the small intestine and caeca increased 43.2 and 28.2%, respectively, relative to those fed the M-S diets. The effects of M-L diets resulting in greater weight and length of these organs of the chickens found in this study are consistent with the results reported by Brenes et al. (2002), who observed greater relative weight of crop, proventriculus, gizzard and duodenum when they included 30 or 45% whole lupine (*L. albus*) seed in the diet. Similarly, Olkowski et al. (2005) reported increases of 26.8 to 36.7% in the length of the different sections of the small intestine in chickens fed diets with 35% dehulled seed of *L. angustifolius*. Hyperplasia of the intestinal tissue has been interpreted as a physiological adaptation of the chickens to increase absorption capacity and thus maximize absorption of essential nutrients in response to the effect of NSP in the diet (Olkowski et al., 2005). However, the physiopathological relevance of these changes in relation to productive variables has not yet been explained (Olkowski, 2011).

3.6. Conclusions

The total substitution of soybean meal by dehulled lupine seed reduces productive performance of broilers in the starter phase and increases size of the digestive organs. Supplementation with xylanases, proteases and β-glucanases used in this study does not improve digestibility or the metabolizable energy value of maize-dehulled lupine seed diets for broilers, but it does increase the AMEn value in maize-soybean meal diets.

3.7. References

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CONCLUSIONES GENERALES

La semilla de lupino descascarada es una mejor alternativa que la semilla entera para reemplazar a la pasta de soya en dietas para aves debido a la mayor concentración de aminoácidos, proteína y energía metabolizable.

La sustitución total de pasta de soya por semilla descascarada de lupino en dietas con enzimas reduce en un 15% la ganancia de peso de pollos de engorda en la fase de iniciación; sin embargo se puede obtener ganancias de peso similares, a la de pollos alimentados con una dieta a base de maíz-pasta de soya sin enzimas, a las seis semanas de edad cuando la dieta a base de maíz-semilla descascarada de lupino se suplementa con xilanasas, proteasas, amilasas y fitasas.

La sustitución total de pasta de soya por semilla descascarada de lupino en dietas para pollos de engorda afecta la digestibilidad ileal aparente de la dieta, incrementa el tamaño de órganos digestivos y el contenido de humedad de la excreta y empeora las variables de bienestar animal.